

THE
JOURNAL OF
COMPARATIVE PATHOLOGY
AND
THERAPEUTICS.

Vol. XLVI.—No. 1.

MARCH 31st, 1933.

PRICE 4s.

GLOBIDIUM INFECTIONS IN THE SUDAN.

By S. C. J. BENNETT, B.SC., M.R.C.V.S.,

Veterinary Research Officer, Sudan Government.

IN an earlier paper the writer (1927) described what was provisionally thought to be a peculiar sarcosporidium in a horse. Its identification as such was recorded as being open to doubt, partly because the earlier stages of development could not be followed, and partly because, in so far as it was studied, it differed in several respects from typical sarcosporidia. Mounted sections were sent to Dr. Wenyon in London, who reported that the parasite was a globidium—either *G. besnoiti* or a closely allied form.

Since 1927 three fresh cases have come to the notice of the Veterinary Laboratory in Khartoum, two in horses and one in a bull, and observations that appear to justify a further communication have been made.

The particular object of this paper is to discuss the infection from the pathological rather than the parasitological point of view, the latter standpoint being largely evaded on account of the almost complete absence of relevant literature in this country. The systematic position of the parasite cannot, however, be entirely ignored, as it will be shown that developmental stages typical of a globidium have not been found, whereas others, more reminiscent of a sarcosporidium, have certainly been established.

In recording one's observations a brief clinical history and *post-mortem* report on each case will be followed by a single account of the changes seen on histological examination, references to the

individual cases being made therein whenever necessary ; the subsequent discussion will include references to the systematic position of the parasite. It will be necessary for the sake of comparison to include notes on the case described in the earlier paper.

FIRST CASE.

Clinical.—Native Sudan pony gelding, about nine years old, had been mainly in the Nuba Mountains (Southern Kordofan). Owing to its poor condition when first seen by a veterinary officer trypanosomiasis was suspected. Blood smears sent to the laboratory revealed no trypanosomes but many "sarcosporos." At the time of these findings the animal had been sick (to the lay eye) for seven or eight months.

Under observation in the laboratory for a further month the condition was more or less constant. The general appearance was of extreme weakness and dejection—hanging head, drooping ears, thickened eyelids, swollen scurfy legs, and staring dirty coat. All parts of the skin were equally affected, although the eyelids and extremities, owing to the more easily noticed swelling at these points, seemed on casual inspection to be the worst. Pulse and respiration were normal, the conjunctiva was a peculiar brick-red colour with a few petechiae, and the buccal mucous membrane pale and dry. None of the palpable lymphatic glands were enlarged, nor was there any pain in any part of the body, or any obvious irritability of the skin. When forced to move, muscular weakness was obvious, and at the end of a few minutes the horse could barely drag its legs along.

The appetite remained good throughout (green fodder only) but thirst was excessive. Faeces and urine showed no abnormality. The temperature was slightly elevated throughout, irregular generally, but usually between 101° and 103° F. Daily blood examination constantly showed "sarcosporos" but no other abnormality or parasites. Slaughter was decided.

Post-mortem.—The visible abnormalities, apart from the external ones already described, were limited to the laryngeal region and the skeletal muscles.

The mucous membrane covering the whole of the larynx anterior to the glottis, the oral surface of the tip of the soft palate, and the glosso-epiglottic fold were studded with large numbers—many hundreds—of spherical white cysts about half a millimetre in diameter. The greatest density was on the epiglottis, vocal cords and false cords, lessening in a forward direction until on the base and pillars of the soft palate and on the base of the tongue none could be seen. The pharynx was free from infestation except for the tip of the pharyngeal aspect of the soft palate, while the posterior larynx was absolutely free. This distribution seemed remarkable, in that from the centre of density the infestation appeared to spread selectively to structures lined with stratified epithelium but not to those lined with ciliated epithelium. It will be seen in considering Case III that this distribution was accidental ; certainly the type of epithelium has no essential bearing on the spread of the infection.

In regard to the skeletal muscles, these were not parasitised, but were all pale brown in colour and very friable, being obviously in an advanced stage of some form of degeneration.

All other organs and tissues appeared to be quite normal ; samples, however, were taken for subsequent microscopic examination, but apart from one cyst in the oesophagus no further site of infestation was found.



Digitized by the Internet Archive
in 2026



FIG. 1.
General view of scrotal and perineal region of infected horse.



FIG. 2.
Higher magnification of scrotal region of horse in Fig. 1. With a good hand lens.

SECOND CASE.

Clinical.—A bull used as a cattle plague antiserum producer in the Upper Nile Province. (It is quite probable that this bull, as the horse in Case I, had come from Southern Kordofan). Suspected of trypanosomiasis by native assistant on account of poor condition. Blood smears revealed no trypanosomes but numerous "sarcospores." It had already been suggested by Dr. Wenyon, in his report on material from the first case, that the presence of spores in blood smears was not due to a true blood-borne infection but was more probably the result of damage to cysts in the skin—presumably at the site of incision while making blood smears. This was found to be the case; in the bull spores could always be found in blood smears made by nicking the edge of the ear, but in several preparations made simultaneously by direct vein puncture no spores were ever seen. It is nevertheless of practical interest that the first two cases of this infection to come to one's personal notice should have been detected from the examination of blood smears.

The infestation of the skin was limited to the fore quarters, neck and head, the face being particularly bad, with thickened eyelids and scurfy thickened ears. The bull did not show any signs of skin irritation, nor did it alter much in condition during the next three months, over which period it was hyperimmunised and bled for serum. No particular observations were made on it during life, but when ultimately destroyed a particularly careful *post-mortem* examination was made.

Post-mortem.—The only visible focus of infestation, apart from the external lesions already recorded, was the laryngeal region. The infestation was not so intense as in the first case, but there were a few hundred small nodules distributed in exactly the same manner. Apart from visibly infected tissues, samples were taken of tongue, œsophagus, all four stomachs, small and large intestines, liver, spleen, kidney, heart, lungs and skeletal muscles. Microscopic examination of these established no new infested tissue.

THIRD CASE.

Clinical.—Native Sudan pony stallion, aged five years. Originated in the Northern Sudan, but had been in the Nuba Mountains for about ten months. When first brought to veterinary notice no definite information could be elicited as to how long the condition had existed; it was, however, declared that there had been no sign of it before the pony went to Southern Kordofan. No diagnosis was made, and after unsuccessful attempts at treatment with a variety of internal and external medicaments the pony was offered to the laboratory.

The general resemblance to the first case was marked, and diagnosis of the same condition was confirmed by microscopic examination of skin scrapings. The whole of the skin was intensely infested, the eyelids and extremities being visibly thickened. The most striking seat of infection was the scrotal and perineal region, in which the repeated bursting of cysts had caused considerable damage, and the effects, owing to the absence of hair, could easily be seen. Here, the formerly black skin was blotched over with unpigmented areas, some of them of barely visible dimensions, but others of up to two or three inches' diameter. The small ones were doubtless cicatrices at the site of bursting of single cysts, the large areas representing confluences of innumerable such small scars. The general appearance of the area is shown in Figs. 1 and 2. In addition to the cicatrices many small

scabs could be seen, representing, as was easily seen on subsequent microscopic examination, "healing wound" areas at the points at which cysts had recently burst.

The horse was only retained for a few days, during which time respiration, pulse and general functions were normal, the conjunctiva was permanently pale brick red with some petechiae, and the temperature remained fairly steady at about 102° F. No rubbing or scratching was noticed.

Post-mortem.—A far more extensive infestation than in the earlier cases. Although the skin was heavily infested, no cysts could be found inside the mouth or the anus; a fair number were present in the nostrils (thus differing from the earlier cases), but these will receive special mention when dealing in detail with the head.

The subcutaneous tissue was even more heavily infested than the skin, containing countless thousands of spherical cysts, varying in size from the limit of visibility to about half a millimetre in diameter. No single area seemed to be more intensely affected than another, but the most striking site was the scrotal fascia, on account of the relatively large amount of loose areolar tissue, the whole of which was packed with cysts.

The skeletal muscles showed signs of degeneration, but not to so advanced a degree as in the first case.

The abdominal and thoracic cavities, together with all their viscera, were normal. Although on dissecting upwards from the scrotum the infestation could be followed, in rapidly decreasing intensity, for some distance along the tunica vaginalis and the tunica albuginea, it did not reach the peritoneal cavity. The testicle proper was also unaffected.

The tissues of the head and neck were extensively affected. Mouth and tongue were normal. Nostrils and false nostrils showed a moderately heavy infestation which diminished on following the nasal passages upwards, until the septum beyond its lower third, and the turbinate bones, bore very few cysts. The whole of the larynx, soft palate and pharynx were intensely infested, the affected laryngeal area extending to the posterior larynx, and, in rapidly diminishing intensity, along the upper half of the trachea; no cysts could be found in the bronchi. A few cysts were also found in the guttural pouches. The œsophagus, except for its extreme upper portion, was free.

FOURTH CASE.

Clinical.—Native Sudan pony stallion, aged eight to nine years, stationed in the Nuba Mountains of Southern Kordofan. This case, which has been and still is under observation at the laboratory, differs from the others in being at a much earlier stage. The lesions, although present in all parts of the skin, are not yet so closely crowded as to make a study of their individual development difficult. The series of changes has been carefully observed, and in all lesions has been essentially identical, except that the superficial appearances have differed slightly in the hairy and hairless parts. The following is a detailed description:—

In the main body of the coat a small group of not more than 15 or 20 hairs stands out away from the coat. When first observed these are clean and still firmly attached, but in a day or two they become stuck together at their base by a gummy exudate, and can be easily pulled out; if left alone they soon fall out. The skin lesion that remains is so small that it is almost impossible to see it in the body of the coat, and nothing further is observed unless a few *white* hairs (rarely more than five or six) grow through in place

of the fallen pigmented ones. Judging from the large number of skin lesions which develop, and the relatively much smaller number of subsequent white flecks, it is certain that white hairs do not always appear. That hair grows again is quite certain, since if it did not a heavily infected horse would soon be nearly bald, and this does not occur. In the hairless parts there can be no sudden erection of hairs to herald new lesions, and persistent careful inspection is necessary in order to detect them. An elevation, never larger than the head of an ordinary pin, develops. At first this is dry, but after several days or a week or more, depending apparently on how early the first sign was noticed, it becomes moist. Without any further definite change the small moist elevation settles down to become a scab of about one millimetre diameter. When this scab falls off or is accidentally rubbed off, either no sign of the original lesion may remain, or a minute unpigmented spot may mark the site. It is tempting to suggest that if the scab falls off there is no mark, whereas if it is rubbed off before it is ready to fall an unpigmented scar remains. It cannot be said that this has definitely been observed, but it is probable, and is consistent with the much more extensive areas of unpigmented skin that develop in hairless parts of the body.

The horse on which the foregoing studies have been carried out has been under observation in the laboratory for four months, during which period the skin infestation has definitely advanced, but not to a marked degree. Apart from the patient's only keeping in fair condition in spite of unlimited food, the disease appears so far to have produced no systemic symptoms.

Reviewing the four above recorded cases, two points at least appear to be worthy of discussion. The first is that, although horses are used throughout most of the Sudan, the only cases of this condition to have come to notice have originated in the Nuba Mountains of Southern Kordofan; it is also probable that the bovine case had a similar origin. There is not as yet sufficient evidence to assess the economic significance of the condition in this locality, and it is quite possible that other infected districts may be found. The topographical distribution is, however, worthy of provisional note. The second point of interest is the distribution of the parasites within the host. Such evidence as exists is in favour of a primary skin infection, although there is no indication of how this originates. If this is so, clinical observation and *post-mortem* findings suggest that spread from the original site of infection is rather via subcutaneous, or submucous, areolar tissue than a diffusion within the substance of the integument itself; the microscopic findings presently to be described will support this view. Grooming and other forms of friction doubtless cause external re-infections. The most interesting site of infestation is the laryngeal area, especially as the parasites in this region are isolated from those elsewhere. Such a distribution undoubtedly suggests alimentary infection, a view that is supported by the observation that the most intense infestation occurs on the epiglottis and the tip of the soft palate. It is certain that although there is no obvious skin irritation a moderate amount of biting of the

skin occurs ; even normal horses indulge in this habit to some extent. Skin biting was actually observed in all the cases under observation in the laboratory but, although its possible significance in regard to the spread of parasites was not disregarded, a special note of it in one's clinical records would have been misleading, since it was very rarely observed, and was certainly not practised to an abnormal degree. In consideration of the permanent contact between epiglottis and soft palate, which in both horse and ox overlap one another considerably, and the friction between these and adjacent organs at every act of swallowing, there is no difficulty in conceiving that this region may become parasitised as a consequence of very little skin biting on the part of the host.

Finally, it is remarkable, if the parasite under consideration is a globidium, that more extensive infestation of the alimentary tract has not been observed, the more especially as infective material is certainly offered by the alimentary route. Little though the pathogenic range of known globidia has been studied, such evidence as does exist indicates that the alimentary tract, and in particular the small intestine, is a predilection seat.

TWO DOUBTFUL CASES.

Before proceeding to a description of one's microscopical findings, two doubtful cases of the same condition may be briefly noted. The first, in a horse from the Nuba Mountains, was of blood smears sent for examination as to trypanosomiasis. No trypanosomes were found but a few "sarcosporos" were seen. The horse could not be subsequently traced. The second case was a donkey which died in Khartoum ; its history was not known, but trypanosomiasis was suspected as the cause of death. As before, no trypanosomes were found in blood smears but a few "sarcosporos" were seen. Unfortunately the carcass had been destroyed before any further action could be taken.

Neither of these cases warrants a definite diagnosis ; the spores in any case would not be sufficiently characteristic. The cases are merely worth mentioning, but it may be significant that in one of them the material originated in Southern Kordofan.

MICROSCOPICAL OBSERVATIONS.

From the diagnostic standpoint, the microscopic characteristics of the spores and the more advanced cysts are important. The spores, which constitute the only diagnostic material in the living animal, can be studied in skin scrapings from an infected area. Experience has shown that the most suitable technique for obtaining reasonably clean preparations is to scrape at first lightly in order to remove hair and scurf, the first scraping being discarded, and then to scrape deeply. The material from the deep scraping is mixed with a little water and spread out on a microscope slide. Fig. 3 shows the degree

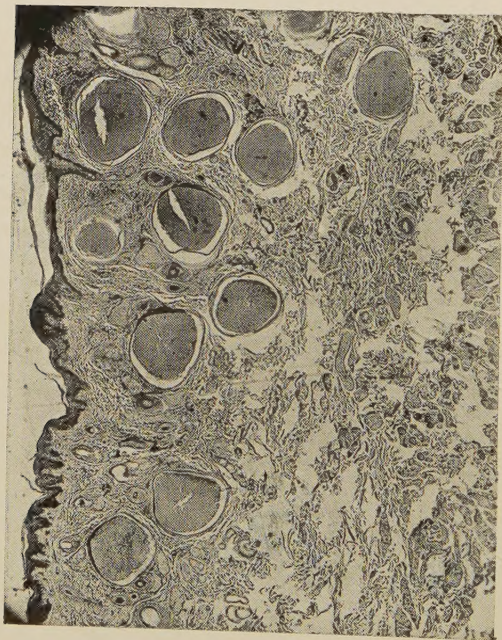


FIG. 3.
Section of horse's skin. Hamalum and eosin, $\times 25$. This photograph is intended to show the density of skin infection only.



FIG. 4.
Spores from a crushed cyst in areolar tissue. Giemsa, $\times 600$. Note that some spores have their chromatin condensed into a central nucleus while others have not.

to which the skin may be infested, and thus the relative ease with which spores should be found. On *post-mortem* examination much better preparations can be made by crushing cysts from the subcutaneous or other areolar tissue. Fig. 4 shows a preparation from a crushed cyst in the scrotal areolar tissue.

The spores can be stained by almost any basic dye, but the best results are obtained with haematein or fuchsin; Romanowsky stains are also suitable. As can be seen from Fig. 4, all spores, even from a single cyst, have not an identical appearance. All are approximately banana shaped and are of the same size, viz., about 10 by 4 μ , but some have sharply defined nuclei while others have not. Moreover, those with well defined nuclei have little chromatic material in the remainder of their body, while the elements without nuclei stain in general more deeply and more uniformly owing to the more even distribution of their chromatin. This phenomenon is so constant that it must have some significance, and it appears to the writer that the most likely explanation is that the concentration or diffusion of chromatic substance is referable to different stages of cell division; multiplication is presumably in progress and all individuals are not at the same stage.

Occasionally a cyst is crushed which is obviously losing in the struggle for existence; instead of the spores being well defined they are misshapen and degenerating; if they had not been obtained by crushing a cyst they would probably not be recognised as organised material of any kind. Fig. 5 shows such material.

In any case the spores themselves, although their recognition is essential for confirmation of clinical diagnosis, are not sufficiently characteristic for identification of the parasite, and they need receive no further attention.

Microscopical examination of organs and tissues, in so far as distribution of parasites within the host is concerned, has already received attention. Such examination has shown that if the parasites could not be detected in any site by the naked eye they were not likely to be found by cutting sections. An odd cyst was in one case found in the oesophagus when none had been seen at the post-mortem examination, but this finding is of no essential importance. In the other tissues examined, apart from degeneration of the skeletal muscles, not only were no cysts detected, but no other abnormality or parasite of any kind was seen, in spite of examining large numbers of representative sections.

The distribution of the parasites from the strictly histological standpoint concerns not so much the organs in which the cysts may be found as the tissue elements with which they are particularly associated or in which they develop. In this sense the distribution can only be negatively described, since in no case has a cyst at any stage of development been found in intimate association with the cellular elements of any tissue. When found in a site containing recognisable areolar tissue, the parasites are found associated with the

latter, but it is not the cellular elements, either fixed or wandering, with which they are associated, since in no case have they been seen except intercellularly. Thus, when cysts are described as being found "in the skin" they are really in the matrix of the areolar or adipose layers of the subcutis or in the intercellular spaces of the more highly differentiated layers; the interfibrillary spaces of the subcutis, however, merge imperceptibly into the lymph spaces of the cutis, and the sites are therefore really identical. Again in the soft palate, to consider another common site, cysts can be seen apparently embedded in the glands and muscles of this organ as well as in the submucous areolar tissue, but a careful examination shows them all to be free; they may be surrounded by gland cells, but there are no signs of their ever having been inside one, and although frequently embedded in muscle tissue none has yet been seen that could be described as "intramuscular" in the sense that it was under the sarcolemma of a muscle fibre.

At this point it is necessary to record one's observations on the structure and development of the cysts themselves. As might be concluded from the remarks in the preceding paragraph, the most convenient material to study is loose areolar tissue, and, of the cases that have come to notice, only one, the third, has had a sufficient quantity of suitable material. Apart from the convenience of distribution in the gross sense, areolar tissue is an ideal site because of its simplicity of structure, which reduces to a minimum the possibility of confusing cells of the parasite with those of the host.

The object of the study has been to follow the parasite through a complete cycle of development, starting from a single spore and working round to the next generation of free spores.

Free spores can be found, although not without some difficulty, on searching sections of areolar tissue with a high power of the microscope. In fixed tissue, they are considerably smaller than in smears from crushed cysts, as a result of the action of the fixative. In consideration of the great numbers of spores in each cyst, it is remarkable how few free individuals can be seen. A further observation of some interest was that, in spite of very lengthy searching no phagocytosis of spores was seen. It appears that, following the rupture of a cyst, the spores rapidly scatter and most of them die. Some, however, survive and these give rise to new colonies. Rare individuals can be seen which have grown to four or five times their original (linear) dimensions, which possess a very clearly marked wall, and which instead of having a well defined nucleus contain several deeply staining granules within a body which itself stains quite readily. Such an element is shown in Fig. 6. It is presumed that these are the founders of new colonies.

Fission or other form of division of a swollen spore has not been seen, in spite of examining a considerable quantity of material, but small groups of one or two dozen normal-sized spores were frequent. Figs. 7 and 8 show a high power and medium power magnification



FIG. 5.
Contents of a cyst undergoing degeneration.
Giemsa, $\times 600$.

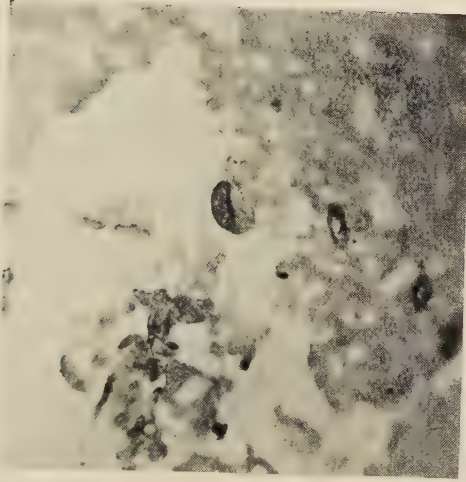


FIG. 6.
Single swollen spore in areolar tissue.
H&E, $\times 600$. Observe the well-marked
outline and the densely staining granules inside
the spore. (The other bodies slightly out of
focus are leucocytes.)

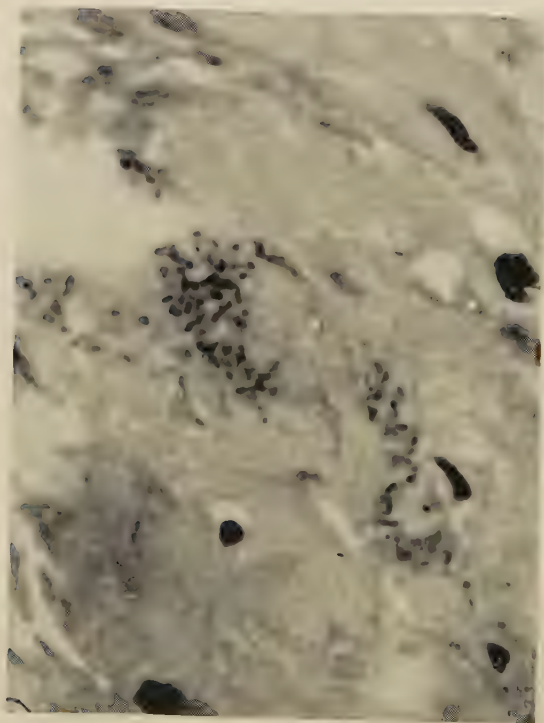


FIG. 7.
Small group of spores free in areolar tissue. Hemalum, $\times 900$.

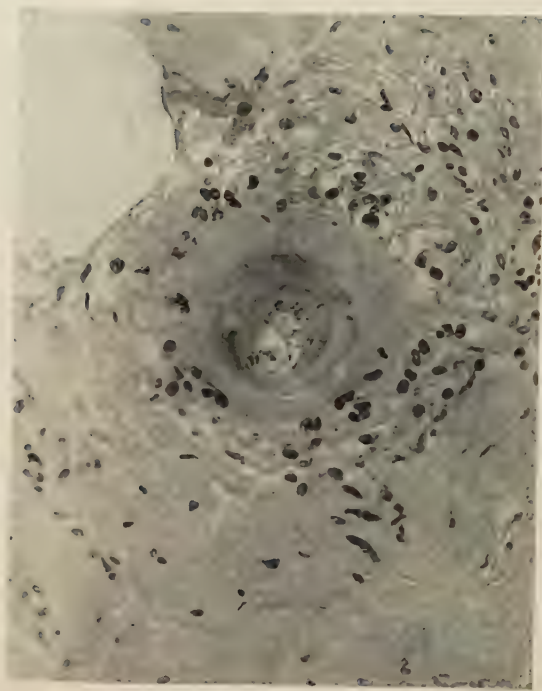


FIG. 8.
Earliest recognisable stage of colony formation in horse's areolar tissue. Hemalum and eosin, $\times 730$. A small number of free spores are separated by a considerable zone of hyaline degeneration from the attacking leucocytes of the heart.

of two such groups. The group in Fig. 7 is the smaller, and lies apparently free in a lymph space, although a certain amount of hyaline degeneration of the adjacent areolar tissue can be seen; the group in Fig. 8 is larger, and is surrounded by a considerable zone of hyaline degeneration. Since every gradation of development up to this point cannot be shown, it cannot be held as proved beyond doubt that the course of events is as suggested above; from the stage shown in Fig. 8, however, there can be no doubt, since throughout the remainder of its development the colony is completely cut off from the host's cells by a well marked zone of hyaline degeneration.

The earliest stage which can justifiably be described as a colony is a small group of spores which is being attacked by the host's leucocytes and which is defending itself undoubtedly by the secretion of a toxin which produces the death and the hyaline degeneration of its attackers. This stage is shown in Fig. 8. The field shown is from a series of sections through the colony, and is selected from approximately its middle; above and below the colony fades into groups of leucocytes only. It is to be noted at this stage that the leucocytes are apparently all monocytes. It is also particularly to be noted that the spores have no suggestive orientation.

From this point the changes are those of a struggle between host and parasite, and the development of the latter cannot be adequately appreciated without simultaneously considering the host's reactions thereto. Broadly considered, the host attacks with leucocytes and fibroblasts in the orthodox manner, while the parasite, under cover of a counter-attack with some kind of toxin which kills and degenerates the attackers, builds a multinucleate capsule around the growing colony.

The first essential change in the parasite is the appearance of first one large nucleus, and later other nuclei, on the edge of the colony. This nucleus is formed by the parasites, which at the site of its formation crowd together so closely that they cannot be individually distinguished. At this point a diffuse, lightly staining nucleus containing a few deeply staining granules can soon be distinguished. Even after the body can be recognised as a separate nucleus it continues to be intimately associated with the spores constituting the colony. At a slightly later stage it loses this close relationship and becomes more definite in outline, although it is still rather diffuse and stains lightly. Fig. 9 shows two nuclei in an early stage of development at the edge of a colony. The more clearly defined one (b) is completely separate, while the less clearly defined one (a) is still associated with the elements of the colony. It is necessary to enlarge somewhat on this observation. First of all, it establishes with certainty the fact that the nuclei under consideration are part of the parasite and not part of the host. *A priori* this would appear certain from a knowledge of the immediately preceding stage of development, of which Fig. 8 is a typical picture. Here the parasites are seen surrounded by a barrier zone of hyaline degeneration which

the host's cells cannot pass ; the actual observation of nuclei being formed by the parasites places the matter beyond doubt. It is unfortunate that the section photographed for Fig. 9 was not a very good one. It had to be used, however, because it was the best one obtainable for illustrating nucleus formation, in that the section was through the centre of two nuclei at slightly different stages of development ; many better fields containing one nucleus only were seen.

Having seen the nuclei develop, it is permissible to anticipate future development and consider what is their function. As will be seen when the later stages of development are described, they appear to be concerned solely in developing an envelope around the colony of parasites, since at no subsequent stage is any association seen between them and the colony elements.

At a slightly later stage the colony has grown, and the contained spores have become more numerous. In spite of this increase in numbers, there is no suggestive arrangement of the spores, or any other appearance to indicate that they have undergone any but the simplest form of division. The host having apparently failed to phagocytise, the parasites next attempts to cut off by the laying down of fibrous tissue. This effort is, however, only successful to a very small degree, since only a small amount of fibrous tissue is made, and it becomes degenerated as soon as it is laid down. It is certain that the toxin which has already been shown to interfere with phagocytosis is equally obstructive to fibroblastic activity. Thus in colonies at the stage under present consideration an additional zone appears, namely, a narrow layer of semi-degenerate fibrous tissue external to the zone of hyaline degeneration already present. The fibrous structure is never very clear, even by van Gieson's method of staining. Fig. 10, which is at the same magnification as the photographs of the two preceding stages, shows a slightly larger colony, containing a larger number of the typical spores, still, it is to be noted, without any suggestive arrangement. Nucleation of the envelope is still proceeding, and the two zones, of hyaline degeneration and fibrous tissue, can easily be seen between the parasites on the one hand and the general cellular reaction of the host on the other.

The ultimate result of the struggle is that the successful parasites complete the nucleated envelope around the colony. A few colonies succumb, and the debris is cleared up by leucocytes. As soon as the envelope is complete, the struggle between host and parasite ceases ; there is apparently no reason for its continuance since the colony, by having made its own envelope, has achieved what the host has been attempting with its fibroblasts. Although the colony continues to grow and the spores to multiply, toxin production ceases, the zone of hyaline degeneration is absorbed, and no more fibrous tissue is laid down ; such fibrous tissue as has previously been made, however, remains and ultimately constitutes the tensely stretched outer wall of the mature cyst. Leucocytary reaction also ceases. Portions of

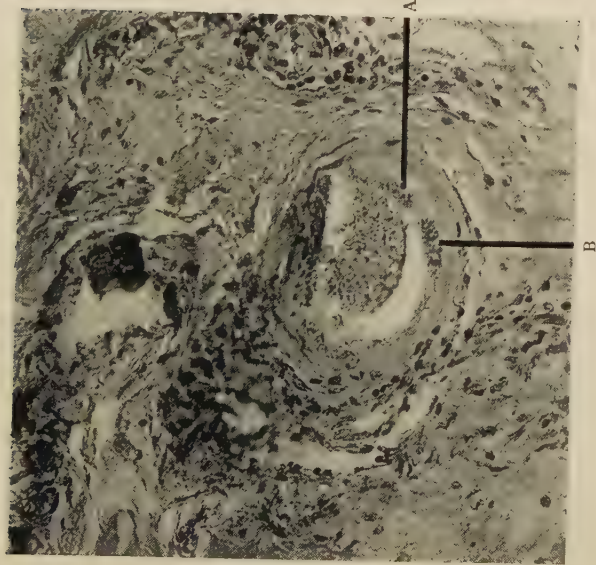


FIG. 9.

A slightly more advanced stage of colony development than that shown in Fig. 8. Hamalium and eosin, $\times 230$. Two nuclei are appearing at the edge of the colony, the younger one (*a*) being still associated with the colony elements while the more developed one (*b*) is quite separate.

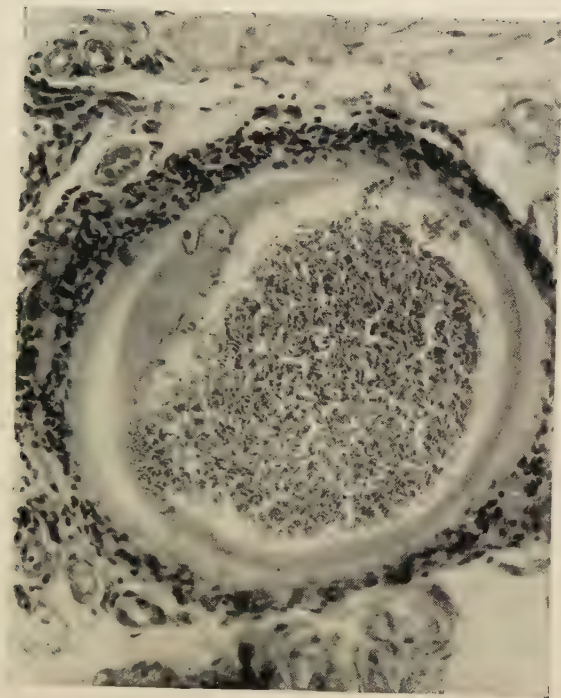


FIG. 10.

A more advanced stage of development than that shown in Figs. 8 and 9. Hamalium and eosin, $\times 230$. The colony is larger, and a layer of fibrous tissue is beginning to appear external to the zone of hyaline degeneration.

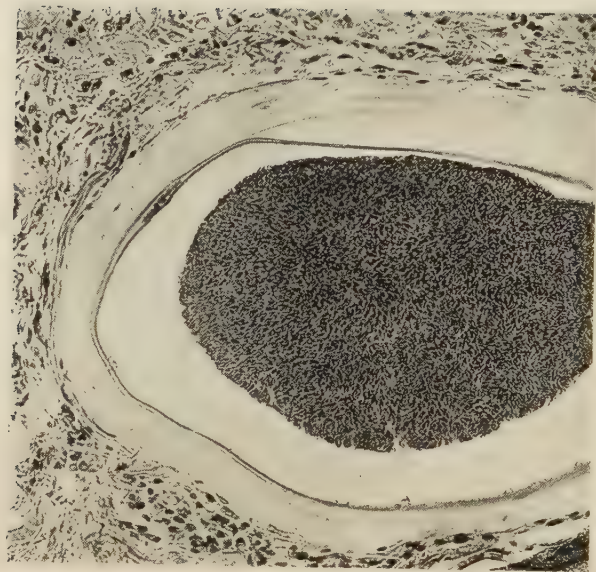


Fig. 12.
A fully mature cyst. Haemalum and eosin, $\times 270$. The cyst wall now consists entirely of the inner nucleated envelope, supported externally by fibrous tissue. Owing to accidental separation the double contour of the cellular envelope can be clearly seen.

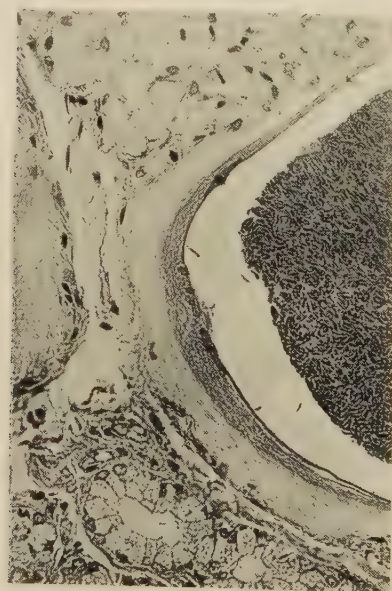


Fig. 11.
Portion of a cyst in an advanced stage of maturity. Haemalum and eosin, $\times 270$. The true envelope and its nuclei are stretched and flattened. The fibrous layer external to the zone of hyaline degeneration is very definite, while the hyaline zone has been largely absorbed. Note particularly the absence at this stage of cellular reaction on the part of the host.

two cysts in an advanced stage of maturity are shown in Figs. 11 and 12. In the former the multinucleate nature of the true envelope is demonstrated by the inclusion of two nuclei in the plane of the section. These have now become much more condensed and flattened, and the cytoplasm associated with them has developed, from an ill defined mass in the zone of hyaline degeneration, into a very sharply demarcated thin envelope. A small amount of hyaline material still remains between this envelope and the surrounding layer of fibrous tissue. The chief interest in Fig. 12 is that the cellular envelope has accidentally become detached from the outer fibrous layer, thus allowing its double contour to be easily seen. In both these photographs attention is drawn to the complete cessation of cellular reaction on the part of the host.

All that now remains, in order to complete the cycle, is for the cyst to burst. It is remarkable that, in spite of examination of a very large amount of material, no cyst has been detected in the act of bursting. The difficulty of finding a bursting cyst was noted when recording the first observed case in 1927. Although in that case very early stages of development were not seen, it was nevertheless observed that as the cysts matured they tended to move towards a surface. This has been confirmed, but the reason for it is not clear; the cysts are not motile, and the more mature the cyst the less is the host's cellular reaction to it. It can only be assumed that pressure effects are responsible for such movement as occurs. All cysts, moreover, do not reach a surface before maturing and bursting, thus showing that arrival at a surface is not essential; in fact internal bursting is largely responsible for the spread of infection within the host.

Regarding the actual mechanism of bursting of a cyst, here again there appears to be no explanation but pressure, in this case from within the cyst. It certainly is a very rapid process, as also is the subsequent scattering of the freed spores, to which reference has already been made.

Many recently ruptured cysts have been seen, both in the depth of the host's tissues and at an epithelium. Fig. 13 shows one that has burst in the depth of the scrotal areolar tissue. It was, before bursting, a mature cyst, as is shown by the well marked fibrous outer wall. Leucocytes are swarming to the spot to clear up the debris, and a few have penetrated to the interior of the empty case. A point of secondary interest may here be raised: Comparison of this photograph with those of earlier stages shows the host's cells to be much more definite and deeply stained; these cells are dealing with a dead cyst instead of with a vigorously developing colony, and are thus not being submitted to the effects of a destructive toxin. Fig. 14 shows the site at which a cyst has recently burst through the tip of the soft palate; there are in addition a few other cysts in the deeper interstitial tissue, including one rather unique individual in which the cellular envelope has formed a septum across the middle.

The remaining histological considerations concern the host only. They may be summarised by stating that wherever a cyst has burst, whether internally or at a surface, a series of changes usually described as a "healing wound" occurs. At a surface this process is so well known as to need no further description, but in the depth of areolar tissue the repair process is essentially the same. Actually, the most noticeable feature of infested areolar tissue, apart from the parasites, is the great vascularity; in fact it is rather loose scar tissue than simple areolar tissue. Leucocytes of all types are frequent, and great care must be exercised in order not to misinterpret their presence. It has already been shown that the cell which essentially attacks the growing colonies of parasites is the monocyte. The cells which clear up the debris are of all types, but include many polynuclears. A few eosinophiles may be present, and owing to their bold appearance may at first sight give the impression of being more numerous than they really are. Cells other than monocytes in the vicinity of a cyst, and particularly of a fairly mature cyst, should not necessarily be considered as directly concerned in that position. Seeing that the essential cell in that position is a monocyte, the other types are more likely to be features of the diffuse scar tissue associated with repair.

Finally, it is necessary to consider the toxin which the parasites emit. In the immediate neighbourhood of developing cysts the effects have already been described in some detail. It must be noted, however, that similar effects are appreciable, although in less degree, at considerable distances. In heavily infested areolar tissue the whole mass is in a state of partial hyaline degeneration. Further, as recorded in the *post-mortem* notes on the first and third cases, when the infection is relatively advanced the whole of the musculature of the body may become affected. Microscopical examination of many samples of skeletal muscles has confirmed a state of hyaline degeneration in them, although in no case have they been parasitised.

Before proceeding to discussion, attention may again be drawn to the fact that the development of the parasite in its earliest stages was studied in one case only—Case III—the reason being that it was only in this individual that an adequate quantity of sufficiently infested areolar tissue was available. It is certain that the same development occurred in the other cases, but the later stages only could be recognised; these were identical with the later ones seen in Case III. The fact of only being able to find late stages of development in some cases will receive attention in the discussion.

DISCUSSION.

It must immediately be understood that no claim is being made to the discovery of a new parasite. The organism under present discussion conforms closely, if not identically, to one which has been less fully described by a number of observers. It seems to have been first described in 1912 by Besnoit and Robin as a sarcosporidium,

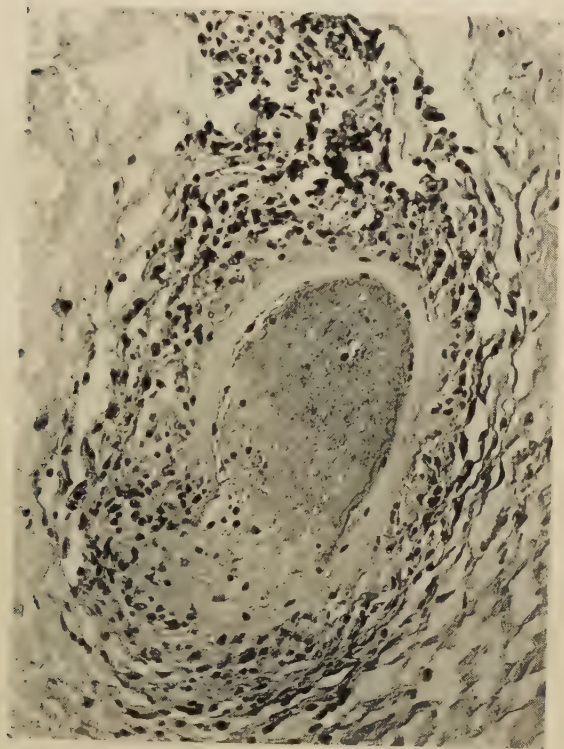


FIG. 13.
A recently ruptured cyst in the depth of areolar tissue. Hæmalum and eosin, $\times 230$. The cyst is being cleared up by the host's leucocytes.

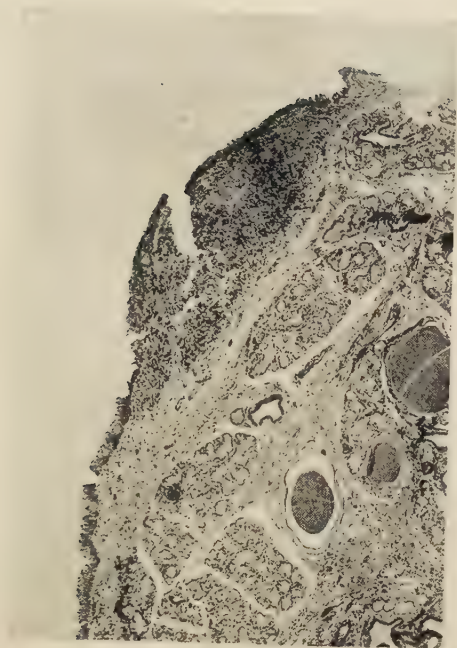


FIG. 14.
Site of recent rupture of a cyst through the tip of the soft palate. Hæmalum and eosin, $\times 40$. The "healing wound" is typical.



later in that year being named *Sarcocystis besnoiti* by Marotel. Franco and Borges (1916) studied what was almost certainly the same organism and suggested that a new genus, *Besnoitia*, should be created for it. In 1920 Nöller concluded that it should be transferred to the genus *Globidium*. The name under which the organism at present passes is therefore *G. besnoiti*. There is thus little doubt about the identity of the parasite which forms the subject of this paper but, in consideration of the mode of development that has been observed, one is forced to conclude that there is still some doubt as to its classification, a doubt, moreover, that is not dispelled by the very vague definition of the genus *Globidium*.

It appears that the recognition of globidia depends on observation of the earlier stages of development, since in the mature stages they closely resemble sarcosporidia. Such literature as is locally available, however, describes developmental forms quite unlike those observed in the parasite under discussion. For example, Wenyon (1926) in collating existing information quotes Chatton's studies in 1910 of *G. gilruthi* at some length. The latter's opinion, to quote Wenyon, is that the cyst wall, which he describes as having only one nucleus, "represents the remains of a very much hypertrophied cell* which may be connected by a kind of neck with the connective tissue. Within the mature cyst is a mass of spores. . . . In cysts which have not completed their development there occur spherical bodies with numerous nuclei arranged over the surface. Portions of cytoplasm, each with one of the nuclei, then grow out from the surface as pointed buds, which gradually assume the character of spores. The latter remain attached to the residue of cytoplasm till they break loose and are scattered within the cyst." Gilruth's (1910) original photographs and Chatton's illustrations (Fig. 331 in Wenyon) support this opinion. To quote Wenyon again: "The various species of *Globidium* agree fairly closely with that of the sheep described above."

In a recent paper Henry and Masson (1932) describe a parasite from a camel which they propose as a new species, *G. cameli*. The paper includes general considerations on the genus *Globidium* and is supported by a number of microphotographs. From the standpoint of the present discussion the interest in Henry and Masson's paper lies in the fact that their parasite, which apparently differs from other species of *Globidium* only in certain measurements, is considered to be typical of the genus.

They propose that the genus should be defined as follows:—"Coccidies volumineuses, dont le développement comporte, d'une part, des oocystes de grandes dimensions, à paroi épaisse (formée par la fusion de gros corpuscules ou globules réfringents), d'autre part, de formes de reproduction endogène qui acquièrent une grande taille." This (?) definition certainly agrees with the description of their parasite, but neither this description nor the microphotographs

* It is not stated whether this cell is considered to be part of the host or part of the parasite.

supporting it could be applied to any stage of the organism studied in the Sudan. It may further be mentioned that Henry and Masson describe their parasite as developing inside one of the host's cells, whereas the organism studied by the writer is intercellular throughout.

In the case of the parasite described by Besnoit and Robin, and by Franco and Borges, conversely, there is little difficulty in identifying it with one's own. The observations are virtually identical, and it is only some of the inferences which differ. The external cyst membrane of Franco and Borges, which they recorded as structureless, is now shown to be semi-degenerate fibrous tissue, or, assuming that they saw even earlier stages, it may have been the zone of hyaline degeneration itself. Their internal membrane, with multiple nuclei resembling those of endothelium, conforms exactly to the more advanced stages in which the nuclei of the envelope have become flattened so as to appear fusiform in section. The spores, moreover, are so clearly identical as to call for no comment. Franco and Borges described no sporoblastic or schizogonic multiplication of spores, and in one's own studies nothing of the kind was seen. In fact, in order to correlate observations in their entirety, one has only to realise that these investigators were limited to a study of the relatively advanced stages of development of the organism.

From the foregoing brief discussion, one may be permitted, in spite of the small amount of literature consulted, to suggest that the organism now known as *G. besnoiti*, including in this species the parasite studied in the Sudan, is probably not a globidium, or alternatively that the definition of the genus *Globidium* needs redrafting. The decision must be left to those better qualified to make it, but it seems that the species *Sarcocystis besnoiti* might still be considered good.

To continue the discussion along more purely pathological lines, there is no doubt that the infection constitutes a definite pathological entity. Its occurrence in horses appears to have been first recorded by the writer in the Sudan in 1927 (as a sarcosporidiosis) and, so far as is known, it has not since been recorded in horses elsewhere. Up to the present, moreover, the infection has in this country only been definitely established in Southern Kordofan, and even in this limited area its economic significance has not yet been assessed. Veterinary control of this area has until recently been practically non-existent; it is at present being developed, but equine practice is still chiefly confined to officially owned animals. Inquiries that are now being made among the horse-owning tribes tend to show that they know the disease quite well, and can so far differentiate it from mange or ringworm as to have given it a separate Arabic name. One tribe, which not many years ago owned 600 to 800 horses, has now less than 50, and it is said that this disease has caused the losses.

It is inadvisable to develop this discussion further in the absence of a more complete survey. The studies recorded in this paper will at least serve as a base to which further information may be referred.

From the standpoint of the field worker the records of outward symptoms and the knowledge that spores can be found with relative ease in skin scrapings should ensure correct preliminary diagnosis. *Post-mortem* features and information as to distribution of parasites within the host are still open to much amplification, but are already sufficiently definite for confirmation of clinical diagnosis, while from the laboratory standpoint the histology has been recorded in sufficient detail to ensure the recognition, if not the classification, of the parasite.

ACKNOWLEDGMENTS.

1. To Capt. J. Going, Sudan Veterinary Service, for preliminary notes on Case I.
2. To Capt. H. B. Williams, O.B.E., Sudan Veterinary Service, for notes on Cases III and IV, and for much information from Southern Kordofan, only part of which is included in this paper.
3. To Mr. A. Marshall, Wellcome Tropical Research Laboratories, for the microphotographs.

REFERENCES.

- Bennett, S. C. J. 1927. A peculiar equine sarcosporidium in the Anglo-Egyptian Sudan. *Vet. J.*, Vol. 83, p. 297.
- Franco, E. E., and Borges, I. 1916. Sur la sarcosporidiose bovine. *Arp. do Inst. Bact., Camara Pestana.*, Vol. 4, p. 269. Orig. not seen, but absts. in (a) *Trop. Dis. Bull.*, 1916, Vol. 8, p. 435, (b) *Trop. Vet. Bull.*, 1916, Vol. 4, p. 95.
- Gilruth, J. A. 1910. Notes on a protozoan parasite found in the mucous membrane of the abomasum of a sheep. *Bull. Soc. Path. Exot.*, Vol. 3, p. 297.
- Henry, A., and Masson, G. 1932. Considérations sur le genre *Globidium*. *Globidium cameli* n.sp., parasite du dromadaire. *Ann. de Parasitol.*, Vol. 10, p. 385.
- Wenyon, C. M. 1926. *Protozoology*, Vol. 1, p. 769. London: Baillière, Tindall & Cox.

NECROBACILLOSIS IN RECENTLY CAPTURED ANTELOPE IN UGANDA.

By R. W. M. METTAM, M.SC., M.R.C.V.S.,

and

J. CARMICHAEL, B.SC., M.R.C.V.S.

ALTHOUGH it is generally known that many species of wild antelope are from time to time decimated during an epizootic of Rinderpest, very little is really known about other diseases from which these creatures suffer in a state of nature.

Knowledge of the part played by game in the maintenance of trypanosomes and other blood protozoa is still, unfortunately, very meagre, but nothing is known as to whether any mortality ever results in Africa from those bacterial diseases that are responsible for mortality amongst indigenous cattle.

In the present paper a disease due to the bacillus of necrosis will be described as it affected freshly captured antelope which were obtained from several different parts of the Protectorate for trypanosomiasis research. It would appear that these unfortunate animals contracted their fatal illness during the first days of captivity, and that the infection was contracted at the laboratory.

The bacillus of necrosis is widely distributed through the tropics and subtropics of Africa, and causes lesions in the domestic animals which agree in every respect with the condition described in modern textbooks as necrobacillosis.

So far as the authors can ascertain from the literature at hand, the organism has never been observed to be responsible for any disease amongst African game.

Footrot, disseminated liver necrosis, and buccal necrosis of calves are very prevalent in Tropical Africa, and recently a fowl was brought to this laboratory suffering from an extensive necrotic lesion of the pad of the foot in which the organism was present.

In Southern Africa, Quinlan, Steck, and Robinson¹ have described a fatal gangrenous pneumonia amongst horses, and in the lung lesions they found the necrosis organism in great numbers. Although they were unable to transmit the disease by cultures of the organism, they had no doubt that it played an important role in the pathogenesis of the disease.

It is worthy of note that Nieberle and Cohrs² have recorded necrobacillosis of the liver of the roedeer in Germany, but other veterinary textbooks are silent on the question of the susceptibility of game.

In the present paper a short paragraph will be devoted to the bacteriological investigations carried out to determine the exact

flora in the buccal lesions, but many attempts to isolate *B. necrophorus* in pure culture proved abortive. This failure is in accord with the experience of many others who have worked with this organism.

Probably the most useful description of the biology and characteristics of *B. necrophorus* is given by Weinberg and Ginsbourg³ in their recent brochure on anaerobes, to which those interested should refer for fuller details.

INCIDENCE OF THE DISEASE AT THE LABORATORY.

A total of some 50 head of game, comprising many species, was received at the laboratory during the early months of 1932. Fatal cases of buccal necrosis were observed in nine of them, viz. seven common duiker (*Cephalophus species*), one oribi (*Montana Cottoni Cottoni*), and two reedbuck (*Redunca Redunca Wardi*). All these animals died, including one reedbuck which recovered after a severe illness lasting two months, only to relapse for some unapparent reason and succumb. The mortality was thus extremely high. All animals, including those affected, were bottle-fed on cow's milk; they were less than three months old and were of both sexes.

On account of lack of accommodation some of the animals were housed in a wood-and-iron shanty and others in an old stable, in both of which experimental cattle and sheep had been constantly kept for some years. Unfortunately, both these buildings were far from ideal hygienically, as during original construction wooden partitions had been used and they had inevitably become thoroughly saturated at their bases with urine and excreta. However, both these places were renovated and disinfected, every effort being made to render them suitable and comfortable for freshly captured young game.

The greater number of the wild animals were placed either in an open air paddock or in a newly constructed stable, which had been built on most modern hygienic lines. No deaths from buccal necrobacillosis occurred in either of these places, the disease being entirely confined to the animals in the other buildings. There seems to be little doubt that the organism responsible for the deaths must have been associated with insanitary conditions, and it seems feasible that the organisms persisted in great numbers in the excreta-sodden woodwork of the older stables. It is well known that calf diphtheria, a serious disease of sucking calves due to the bacillus of necrosis, often exists as a stable infection and as such may be responsible for considerable loss of life (Nieberle and Cohrs², Huttyra and Marek⁴, and others). The similarity between calf diphtheria and the buccal necrosis of these game animals is so great as to leave no doubt that they are really the same condition.

It is of interest, however, to keep in mind the fact that calf diphtheria has not been diagnosed up to the present amongst young cattle at this institute. Possibly native bred calves, which are often

subjected to life under most adverse hygienic conditions, have developed a considerable immunity against organisms of the necrophorus type. New born calves and lambs placed in the pens in which the young game died failed to develop the disease. Since game have no longer been placed in the old stables no further cases of the disease have arisen.

Certain predisposing causes undoubtedly existed. Some of the young animals were naturally extremely nervous and struggled while being handled and bottle-fed during the early days of captivity. It is therefore likely that they injured their mouth parts at this time, thereby facilitating the penetration of the necrophorus organisms, which have a predilection for buccal tissues. At autopsy it was observed that all cases were still cutting their back molar teeth, thereby offering additional avenues of entrance for the organism into the body.

PERIOD OF INCUBATION AND SYMPTOMOLOGY.

The earliest case to show symptoms of stomatitis was a female duiker within 14 days of her arrival at the laboratory. The majority of animals first evinced signs of illness between 17 and 21 days after admission, though it is possible that the lesions were present a few days before clinical symptoms declared themselves.

The first sign of illness was a slight swelling involving one side of the face and the intermaxillary region. This steadily increased in size, was hard and firm to touch, and was most painful along its periphery. No heat was observable, and the swellings, which might be as large as the palm of the hand, showed no tendency to point or burst. Salivation was greatly increased and the very noticeable foul odour of necrotic tissue emanated from the mouth. The animals became greatly depressed and hid away in a corner out of which they were very unwilling to move. At first the appetite was not impaired, but as the disease progressed it became more and more capricious, until finally even liquid food was refused altogether. All animals on admission to the laboratory were in good condition, but as the disease became more and more pronounced they became thinner and thinner, until, before death, emaciation was very marked. Examination of the mouth was fiercely resented, but in all cases the mucosa was more or less deeply injected and necrotic areas of various sizes could be seen on the tongue, cheeks, and hard palate. When the swellings became very pronounced it was extremely difficult to open the mouth at all. Very frequently there was a profuse watery nasal discharge, and a slight lacrymation from one or both eyes. Respirations remained normal except in the one case that developed acute pneumonia. As it was believed preferable to leave the animals alone as much as possible, no attempts were made to take temperatures daily, but the general increase of warmth of the body surface showed a moderate degree of fever to be present. Prior to death the animals

became uneasy and their expression was haggard and anxious. For the last 24 hours of life there was deep coma, terminating in death. Throughout the disease the animals were inclined to be constipated. Nothing unusual was noted about the urine.

Duration of illness varied from two to four weeks except in the case of one reedbuck in which the acute symptoms slowly subsided. After three months, however, there was a sudden and quite unexpected recurrence of the symptoms, and the case terminated fatally within 14 days. Although it is impossible to give any explanation for this relapse, it seems possible that the lesion was ruptured during a fight with other animals.

In an oribi the disease was complicated in its final phases by acute pneumonia, which presented all the usual classical symptoms. This animal was only sick for 14 days, which was the shortest course of all. It is most likely that the disease was hastened to a fatal termination by the complicating pneumonia.

PATHOLOGICAL ANATOMY.

Except in the oribi, the lesions were confined to the buccal cavity and naso-pharynx. In the oribi there were extensive pneumonic and marked vascular changes in the other parenchymatous organs and intestines. The visceral lesions of this case will be described separately.

In every animal the cheek, hard palate, soft palate and tongue were affected, while in the oribi the main lesion extended into the pharynx, especially around the posterior choanae, and to the vicinity of the anterior pillars.

The lesions were always multiple. They varied in size from about 1 centimetre in diameter up to a diffuse necrotic area as much as 7 or 8 centimetres across its greatest width. As might be expected, they were most irregular in shape. The largest was met with in the oribi and involved the cheek, gums, hard and soft palate, tongue and fauces over an irregular area measuring 10 centimetres across its greatest diameter. The smaller lesions were confined to the tongue, one or both cheeks, the anterior half of the hard palate, or the lateral walls of the pharynx. In all, no matter what the size of the lesion was, the structure was the same, viz :—(1) an outer peripheral zone, reddish-purple in colour, which acted as a kind of capsule; and (2) an inner zone completely filled by a homogeneous necrotic mass, greyish-white in colour, caseous or lardaceous in consistence, and extremely foetid. Underneath the tongue, in the cheeks, and in places where there was a good deal of loose connective tissue, there was a very noticeable amount of greenish-yellow gelatinous material infiltrating along the intermuscular septa. This semi-fluid œdema had a very foul odour. The osseous hard palate, the coronoid process of the mandible, and the alveolar processes of mandible and maxilla in the areas where necrosis was severest, showed no signs beyond

marked stasis of blood in the periosteal capillaries. In the oribi, two duikers and the one reedbuck, the tongue was so severely necrosed that healthy tissue could only be detected in the anterior free portion. Adjacent lymphatic glands, *i.e.* the mandibular, sub-parotid, pharyngeal, and superior cervical, were much swollen and markedly hæmorrhagic. All were quite free from any metastatic caseous lesions.

The skin over the facial swellings was partially or completely depilated and covered by a straw-coloured exudation. In no case was there any external suppurating sinus or ulcer.

In the reedbuck that relapsed after appearing to be convalescent the lesions were the same as those described above. The sub-parotid and pharyngeal glands were greatly enlarged and very hyperæmic. The necrotic lesion had ruptured into the mouth and there was a considerable quantity of foul-smelling necrotic material in the pharynx and rumen. No evidence could be obtained of any injury which might have caused the lesion to burst. As in all other cases, death was apparently due to toxæmia.

In the oribi the following additional lesions were present, viz :— in the serous cavities there was a large increase of straw-coloured fluid, which, in the cases of both pleural and pericardial cavities, was deeply blood-stained. The whole of the right parietal pleura, the pleural covering of the right lung, and both faces of the pericardium were covered by an easily detachable, soft, greenish-brown, fibrinous deposit, removal of which exposed a deeply injected serosa. Both parietal and visceral deposits were connected by easily broken down adhesions. The whole of the anterior and cardiac lobes and the front half of the main lobe of the right lung showed very severe changes. The affected parts of the lung were solid, soggy in consistence, and purplish-brown in colour. Lobulation was not at all clear. On section these areas were swollen, hepatised, reddish-purple in colour but with a distinct greenish tinge. Scattered throughout the consolidated lobes were numerous small pea-like foci full of caseous material similar in appearance to the material found in the mouth lesions. Pieces of pneumonic lung sank immediately when placed in water. The bronchial mucosa was petechiated and the lobes contained much froth and catarrhal material.

The right bronchial lymphatic glands were swollen, œdematous, and hæmorrhagic. The left lung was a bright pink colour, somewhat doughy in consistence but free from consolidation.

The pericardium showed an extension of the lesions which involved the right mediastinal pleura, and the epicardium was obscured by a considerable amount of flaky fibrinous material, yellowish-brown in colour. Floating in the pericardial fluid was a small quantity of the same material. The myocardium itself appeared normal.

The kidneys and spleen contained an excess of blood and were very swollen. The renal cortex showed very pronounced streaky red hæmorrhages.

The mucosa of the small intestine was deeply hæmorrhagic throughout, but the caecum, colon and rectum appeared to be normal.

The liver was much swollen and had thick blunt edges. Generally of a deep purple colour, it showed here and there light putty-coloured areas. Lobulation was indistinct. The cut surfaces had a homogeneous parboiled appearance and showed coloured areas similar to that noticed on the surface. Consistence was very soft and the organ contained an excess of blood which, on lightly squeezing the organ, oozed most profusely from the portal system.

No other changes were noticed in any of the other organs.

HISTOLOGY.

Material for sectioning was fixed in either Bouin or 4 per cent. formol saline. Both paraffin and frozen sections were employed. For the demonstration of bacteria best results followed staining by Helly's modification of Giemsa.

All the buccal lesions show the same histological changes. The centre of the lesion is occupied by a homogeneous tissue which stains feebly and shows no definite structure. It is in a state of coagulative necrosis. Here and there are found masses of disintegrating cells and pyknotic nuclei which stain rather deeply with methylene blue and hæmatin. *Necrophorus bacilli* are everywhere, and they may be the only structures made out with any clarity away from the edge of the lesions. The organisms are remarkably pleomorphic, revealing a number of forms and shapes, from stout cocco-bacilli to long wavy filaments 10 to 20 μ long. The most interesting and remarkable changes are to be observed on the very periphery of the lesion where healthy tissues are being attacked and invaded. Here it will be seen that the lesion spreads by masses of *necrophorus bacilli* infiltrating between the cellular elements of the unaffected tissue.

This invasion soon provokes a violent cellular reaction, and large numbers of phagocytic leucocytes, fibroblasts, and epithelial cells appear as a barrier to further progress. Here a deeply staining ring or zone is produced which represents the attempts of the cellular elements to resist further penetration by the bacteria. Just internal to this is a lightly stained zone in which can be seen destroyed tissue and various kinds of organisms in enormous numbers, the *necrophorus bacilli* predominating.

The tissue has already become a structureless matrix, which becomes more and more pronounced the further from the periphery.

On the very edge of the lesion, where active invasion is taking place, *necrophorus bacilli* are found in characteristic bundles arranged close together and forming regular sheaves. In this position the long filamentous forms predominate, while the invasive forms appear mainly to be long beaded bacilli or shorter forms showing no cytoplasmic differentiation. In the necrotic material itself intermediate delicate wavy organisms are chiefly found. Besides *B. necrophorus*

other bacteria are also recognisable, chiefly bipolars and streptococci. Possibly the former are the same *pasteurella* which were always recovered from the heart blood after death, and which proved to be so pathogenic for rabbits and guinea-pigs (see Bacteriology).

Where the part of the mouth involved contains much voluntary muscle tissue, as in the tongue and cheek, or numerous mucous glands, as in the cheek and soft palate, the gradual destruction of these structures can be followed from the time they are first penetrated by *B. necrophorus* to their final involvement in the coagulative necrosis.

In the lung of the oribi the lesions are those of a septic broncho-pneumonia. In the vicinity of the lesser bronchii and bronchioles the alveoli are completely swamped by an œdematous fluid, and in the air cells themselves are great numbers of leucocytes and desquamated epithelial cells and much catarrhal material. The bronchial passages are completely plugged by cellular elements of the same nature, and in them are masses of *necrophorus* organisms. Scattered throughout the pneumonic areas are what appear to be multiple miliary abscesses. These are found to be greatly distended bronchioles filled with purulent material containing immense numbers of *necrophorus* organisms.

The vessels accompanying the bronchial system, as well as those in the interlobular connective tissue, show acute stasis, while the septa are swollen and infiltrated with an œdematous fluid.

The mandibular and pharyngeal lymphatic glands of all cases, as well as the bronchial glands of the oribi, show acute congestion. No micro-organisms can be detected in any of them.

EXPERIMENTAL TRANSMISSION OF THE DISEASE.

The disease could be readily transmitted to calves by inoculation of salivary juices from sick game or of necrotic matter from dead cases. In every instance abscesses were produced at the site of inoculation, whether in the cheek, palate, tongue, lips or gums. Such abscesses took about five to six days to mature and were preceded during the 48 hours after inoculation by hyperæmia at the site of injection. On bursting, a glary, tenacious, greenish-white pus exuded which contained masses of *necrophorus* organisms.

All experimental calves made an uninterrupted recovery, though while the abscesses were developing there were symptoms of acute stomatitis, general malaise, and a few degrees of fever. All these symptoms rapidly disappeared when the abscesses burst. No permanent lesions were recorded.

New born calves and lambs placed in close contact with sick game failed to develop lesions of the disease. One lamb died after being housed with sick game for 17 days, but no signs of any buccal lesions could be made out at autopsy and the cause of death remained obscure.

RECOVERY OF THE ORGANISM FROM THE LESIONS.

Although sections of the diseased tissues showed enormous quantities of organisms resembling *B. necrophorus*, all attempts at obtaining a pure culture of this organism failed.

Material from the centre and periphery of the necrotic lesion and the retropharyngeal lymph glands was incubated aerobically and anaerobically.

Loeffler's serum slopes, serum agar slopes, serum broth, and deep stabs of inspissated ox serum were used.

The anaerobic cultures were grown in a Wilson's (1928) modification of McIntosh & Fildes jar⁵, in an atmosphere of hydrogen, and also in Buchner's tubes over sodium pyrogallate as recommended by Kelser (1927)⁶. Growths occurred both aerobically and anaerobically.

Aerobically.—A mixed flora consisting mainly of staphylococci, streptococci, and a small Gram-negative cocco-bacillus was obtained from all cultures.

Anaerobically.—(5 days at 37° C.) Cultures of necrotic material produced a characteristic foetid odour of "dead" tissue which was very similar to the smell which emanated from the diseased animal's mouth. Smears showed a small Gram-negative cocco-bacillus, a number of Gram-negative filamentous bacteria, and a sporulating organism which was weakly Gram-positive.

This latter organism was obtained in pure culture as a long slender curved bacillus with round terminal spores. It fermented none of the usual carbohydrates, and proved non-pathogenic to guinea-pigs when inoculated intramuscularly into the hind leg.

The small cocco-bacillus which occurred in both the aerobic and anaerobic cultures proved a species of *Pasteurella* of the septica group.

When necrotic material, as well as cultures, both anaerobic and aerobic, were inoculated into the lower lip of rabbits, the presence of this pasteurella completely negated any attempt to obtain a necro-bacillary lesion as all the animals died from septicæmia within 18 hours.

The anaerobic organism further complicated the attempts to obtain the *B. necrophorus* in pure culture, although in films from the primary anaerobic growth numerous thread-like organisms similar morphologically to the necrosis bacillus were seen.

SUMMARY.

1. A disease of young captive wild antelope is described in which all clinical cases died.

2. The symptoms were those of an intense necrosis of the buccal tissues, great prostration and, in one oribi, acute pleuropneumonia.

3. Bacteriologically the condition was apparently primarily due to the organism *B. necrophorus*, which was present in the tissues in great numbers, though attempts to isolate it in pure culture failed.

4. The disease appeared as a "stable infection" and was only observed in animals kept in wooden premises which had previously been occupied by cattle, sheep and goats. It was not encountered in game animals kept in an open air paddock, or in a newly built stable of modern design.

5. Attempts to transmit the disease to young domestic ruminants by contact with sick game failed, but inoculation of morbid material set up an acute abscess from which the experimental animals recovered.

NOTE.—We wish to record our thanks to the Director of Veterinary Services for his interest in the investigation and for permission to publish this paper.

BIBLIOGRAPHY.

- ¹Quinlan, J. B., Steck, W., Robinson, E. M. 1926. "Necrobacillosis in equines; clinical, pathological, and aetiological studies on an outbreak." 11th and 12th Reports of the Director of Veterinary Education and Research, Part I, pp. 571-616. Pretoria.
- ²Nieberle, K., and Cohrs, P. 1931. *Lehrbuch der Speziellen pathologischen Anatomie der Haustiere*. Jena.
- ³Weinberg, M., and Ginsbourg, B. 1927. *Données recentes sur les microbes anaerobies et leur rôle en pathologie*. Masson et Cie., Paris.
- ⁴Hutyra, F., and Marek, J. 1926. *Special Pathology and Therapeutics of the Diseases of the Domestic Animals*. 3rd authorised American edition.
- ⁵Wilson, G. S. 1928. *Journal of Pathology and Bacteriology*.
- ⁶Kelser, R. A. 1927. *Manual of Veterinary Bacteriology*, pp. 255-6.

THE VIABILITY OF THE VIRUS OF SWINE FEVER IN BONE MARROW, MUSCLE AND SKIN OF PRESERVED CARCASSES.

By T. M. DOYLE.

Ministry of Agriculture Laboratory, Weybridge.

THE first reference to the dangers of offal as a source of swine fever infection appears to have been made by Detmers in 1880 in connection with an outbreak of the disease in Illinois, U.S.A.

Rutherford in 1911, referring to the disease in Canada, also pointed out the risk of introduction through this source.

In 1912 McGilvray brought forward strong circumstantial evidence that swine fever was introduced into Winnipeg through feeding pigs with hotel refuse.

Birch (1917) appears to have been the first to prove conclusively the important part played by infected bone and muscle in the dissemination of swine fever, although such material had been long under suspicion.

Important contributions to this subject have also been made by Magnusson and Holth (1927), Zeller and Beller (1929), and Henry, Seddon and Blumer (1931).

Minett (1927), working for the British Foot and Mouth Disease Research Committee, showed that the virus of foot and mouth disease survived 76 days in the bone marrow of beef and bacon carcasses stored under "trade" freezing conditions, and for 42 days in chilled carcasses. In dry-salted bacon carcasses the bone marrow was found to be infective by guinea-pig inoculation after 43 days; virus could not be demonstrated in the bone-marrow of any bacon carcass which had been in storage for 76 days. Purchase (1931) recorded that the virus of fowl plague survived 287 days in muscle, and 303 days in bone marrow, of "chilled" carcasses.

In experiments recently carried out in this laboratory the virus of "Newcastle disease" of fowls was found to survive for six months in bone marrow and muscle of "chilled" carcasses.

PLAN OF EXPERIMENTS.

The experiments here described were undertaken with the object of ascertaining the viability of the virus in bone marrow, muscle and skin of cured carcasses of pigs killed in the early stage of the disease and before the appearance of clinical symptoms, as this appeared to be a question of sufficient practical importance to a large pig-rearing and bacon-importing country to merit further investigation.

The experimental pigs, which weighed from 50 to 60 lbs., were housed in isolated iron sties situated 30 feet apart. The pigs under

test with the chilled material were kept in one row of sties, while those being tested with the salted material were placed in another row about 100 yards away. A separate attendant was employed for each group. Several healthy pigs were placed in intervening sties in each row as controls against accidental infection, although there were no other affected pigs on the premises. There would not appear, however, to be much risk of the accidental spread of swine fever under laboratory conditions, provided ordinary precautions are observed.

In most of the experiments a control pig was placed in contact with the one under test in order to prove the infectivity of the disease induced and to guard against the possibility of confusion with necrotic enteritis (*B. suispestifer* infection), which is only slightly contagious.

All deaths recorded in these experiments were caused by acute swine fever, the diagnosis being based on the symptoms, post-mortem lesions, and infectivity of the disease induced.

Lesions of Acute Swine Fever.

In order to avoid repetition by describing separately the post-mortem lesions present in each pig, it is proposed to mention briefly the principal lesions of acute swine fever and to note later only those lesions which are not here indicated.

Petechial hæmorrhages are the true lesions of acute swine fever. These may occur on the membranes lining the thoracic and abdominal cavities and on the contained organs; on the intestinal mucous membrane; in the larynx; and on the skin and in the subcutaneous tissue.

The lymphatic glands are invariably enlarged, and show on section either petechiae or a variable degree of inflammation. Pigs occasionally die of acute swine fever without showing lesions other than those of the lymphatic glands.

Lesions generally occur in the cardiac portion of the stomach. These consist of petechiae or superficial ulceration or hyperæmia; in acute cases the normal pink colour of the stomach is changed to a red or purplish-red colour.

The frequency of intestinal ulceration in pigs affected with acute swine fever varies considerably in different outbreaks: it occurred in about one-third of the pigs infected at this laboratory. The presence of ulceration would appear to depend to a considerable extent on the bacterial flora of the alimentary tract, which is in turn influenced by the conditions of sanitation under which pigs are kept, sanitation and feeding being intimately associated in the pig. This would support the general conception that ulceration is produced by the action of certain secondary organisms, but probably only when these are in excessive numbers. It is quite clear, and is indeed generally recognised, that intestinal ulceration is not caused by the filterable virus *per se*.

A point of interest is that extensive ulceration may be present in

the intestines of pigs which have died of swine fever but in which *B. suispestifer* is absent from both the internal organs and the alimentary tract.

Methods of Preservation.

The carcasses employed were preserved in three ways :—

- (1) by chilling ;
- (2) by dry-salting ;
- (3) by smoking.

Chilling.

In this method the carcasses were kept in an ice-box in the cold store. The temperature of the ice-box was recorded morning and evening and varied considerably during the experiments ; the lowest temperature registered was 14° F. and the highest 28° F., but during the greater part of the time the temperature was about 26° F. The “ trade ” freezing temperature is from 10° F. to 15° F., and the chilling temperature from 28° F. to 30° F.

Dry Salting Process.

In this method powdered salt was rubbed into the carcass, which was then placed under salt and left for four days at ordinary temperature before being moved to the cold store, the temperature of which varied between 30° F. and 34° F.

Virus.

The virus employed was obtained from pig No. 190, which was infected by contact with a sick pig at the laboratory. It exhibited typical symptoms of swine fever and died on the eighteenth day. Post-mortem examination revealed the usual lesions of acute swine fever.

Pieces of liver and spleen were ground up, suspended in distilled water in proportion of 1 to 10 by weight, passed through filter paper and then through a Berkefeld candle Mark V, under a negative pressure of 15-in. Hg. The reliability of the filter was controlled by adding 10 c.c. of an 18 hour broth culture of *B. avisepticus* to the liquid before filtration and sowing 5 c.c. of the filtrate in Martin's broth and incubating at 37° C. for one week.

The tests for the presence of virus were made by feeding pigs with samples of the material and in some cases by subcutaneous inoculation. The bones were crushed before feeding and the skin and muscle were passed through a mincing machine.

EXPERIMENT I.

The Viability of the Virus in Bone Marrow.

Chilling Temperature.—Two pigs—259 and 260—were inoculated subcutaneously with 10 c.c. of filtrate (Berkefeld) of an organic suspension of pig No. 190.

These pigs were shot and bled at the height of the temperature reaction, and although they had ceased to feed they were not visibly ill and would have passed an inspection which did not include the taking of temperatures.

Results of Post-mortem Examinations.

Pig 259.—The thoracic and abdominal lymphatic glands were slightly congested. A few petechial hæmorrhages were present on the surface of the lungs and in the caecum and large colon.

Pig 260.—This pig showed lesions similar to those found in No. 259 and in addition some oedema of the wall of the large colon.

Control to Infectivity of the Blood of Nos. 259 and 260.

The mixed blood from these pigs was diluted with nine times its volume of distilled water, and 10 c.c. (1 c.c. whole blood) inoculated subcutaneously into pigs 253 and 264. A control pig, 230, was placed in contact with the inoculated pigs. Pigs 253 and 264 died on the twenty-seventh and forty-third day respectively. Pig 230, infected by contact, died on the twenty-seventh day.

Dry Salted Carcase.—The material for this experiment was obtained from pig 255, which was inoculated subcutaneously with 10 c.c. of the same filtrate as used for infecting pigs 259 and 260. It was shot and bled at the height of the temperature reaction and before the appearance of visible symptoms.

Result of Post-mortem Examination.

Pig 255.—All the lymphatic glands were slightly enlarged. The wall of the large colon showed considerable oedema, and about half a dozen petechial hæmorrhages were present on the mucosa

Control to Infectivity of the Blood of No 255

Blood was collected at time of slaughter, diluted with nine times its volume of distilled water and 10 c.c. (1 c.c. whole blood) was inoculated subcutaneously into pig 265. This pig died on the eighteenth day. A control pig, 234, placed in contact with 265, died on the twenty-seventh day.

Test I.

This was carried out after the material had been in storage for fourteen days.

(a) Chilled carcasses.

(1) *By feeding.*—Two long bones, one from each carcase, were crushed and with the contained marrow fed to pig 277. This pig died on the nineteenth day. A control pig, 236, placed in contact with 277, became very ill but eventually recovered. It was subsequently tested for immunity and resisted, without any apparent effect, a subcutaneous inoculation of virus which killed a susceptible pig in twenty-six days.

(2) *By Inoculation.*—A sample of marrow from the bones used in the feeding experiment was shaken with normal salt solution, filtered through paper, and 20 c.c. inoculated subcutaneously into pig 276. This pig died on the thirty-third day.

(b) Dry Salted Carcase.

(1) *By Feeding.*—One long bone and the contained marrow was fed to pig 282. This pig died on the sixty-third day. A control pig, 239, placed in contact with 282, died on the thirty-sixth day. Two areas of necrosis were present in the larynx of pig 239.

(2) *By Inoculation*.—A portion of marrow from the bone used in the feeding experiment was inoculated subcutaneously into pig 263. This pig died on the nineteenth day.

Conclusion.—The virus of swine fever survived fourteen days in the bone marrow of chilled, and dry salted, carcasses.

Test II.—After Twenty-nine Days.

(a) *Chilled Carcasses.*

(1) *By Feeding*.—Two long bones were crushed and with the contained marrow fed to pig 285. This pig died on the twenty-seventh day. A control pig, 294, placed in contact with 285, died on the twenty-fourth day.

(2) *By Inoculation*.—A sample of marrow from the bones used in the feeding experiment was inoculated subcutaneously into pig 291. This pig died on the twenty-fourth day.

(b) *Dry Salted Carcase.*

(1) *By Feeding*.—One long bone and the contained marrow was fed to pig 284. This pig died on the thirty-eighth day. A control pig, 297, placed in contact with 284, died on the forty-fifth day.

(2) *By Inoculation*.—A portion of marrow from the bone used in the feeding experiment was inoculated subcutaneously into pig 273. This pig died on the eighteenth day. In addition to the usual lesions there was a considerable quantity of yellow serous fluid in the pericardial sac and a large superficial area of necrosis in the caecum.

Conclusion.—The virus of swine fever survived twenty-nine days in the bone marrow of chilled, and salted, carcasses.

Test III.—After Seventy-three Days.

(a) *Chilled Carcasses.*

(1) *By Feeding*.—Two long bones were fed to pig 296. This pig became prostrated by an acute attack of swine fever and showed marked bluish discolouration of the skin, which is usually a premonitory symptom of death. It eventually made a partial recovery but remained emaciated and unthrifty in appearance.

A control pig, 302, placed in contact with 296, became infected but in a milder degree, and it also made a partial recovery. Both pigs were killed on the forty-second day.

Results of Post-mortem Examinations.

No. 296.—The lymphatic glands were much enlarged. There were a few petechial hæmorrhages in the larynx and three "button" ulcers were present at the base of the ileo-caecal valve.

No. 302.—Enlargement of the lymphatic glands was the only change observed.

(2) *By Inoculation*.—A sample of mixed marrow from the bones used in the feeding experiment was inoculated subcutaneously into pig 292. It died on the sixteenth day.

(b) *Dry Salted Carcase.*

(1) *By Feeding*.—One long bone was fed to pig 293. This pig died on the twenty-ninth day.

A control pig, 303, placed in contact with 293, died on the thirty-third day.

(2) *By Inoculation*.—A sample of marrow from the bone used in the

feeding experiment was inoculated subcutaneously into pig 295. This pig died on the fifteenth day.

Conclusions.—The virus of swine fever survived seventy-three days in the bone marrow of chilled and salted carcasses. The bone marrow experiments were discontinued at this point.

SUMMARY.

EXPERIMENT I.—BONE MARROW.

<i>No. of Pig.</i>	<i>Method of Infection.</i>	<i>Method of Storage.</i>	<i>In Storage Days.</i>	<i>Result.</i>
277 }	By feeding	Chilled	14	Died 19th day.
236 }	Control-contact	—	—	Ill, recovered.
276 }	Inoculation	Chilled	14	Died 33rd day
282 }	By feeding	Dry salted	14	Died 63rd day
239 }	Control-contact	—	—	Died 36th day
263 }	Inoculation	Dry salted	14	Died 19th day
285 }	By feeding	Chilled	29	Died 27th day
294 }	Control-contact	—	—	Died 24th day
291 }	Inoculation	Chilled	29	Died 24th day
284 }	By feeding	Dry salted	29	Died 38th day
297 }	Control-contact	—	—	Died 45th day
273 }	Inoculation	—	29	Died 18th day
296 }	By feeding	Chilled	73	Ill, recovered
302 }	Control-contact	—	—	Ill, recovered
292 }	Inoculation	Chilled	73	Died 16th day
293 }	By feeding	Dry salted	73	Died 29th day
303 }	Control-contact	—	—	Died 33rd day
295 }	Inoculation	Dry salted	73	Died 15th day

EXPERIMENT II.

The Viability of the Virus in Muscle.

In view of the statement contained in the Second Progress Report of the Foot and Mouth Disease Research Committee that the virus of foot and mouth disease does not persist in the muscle of infected carcasses after rigor mortis has occurred, it was considered unnecessary to carry out experiments on this aspect of the swine fever problem, but towards the end of the bone marrow experiments interest in the question was aroused by an outbreak of swine fever in which there was some evidence pointing to meat scraps as a possible source of infection. Advantage was therefore taken of the material remaining in cold store and the following experiments were carried out.

*Test I.—After Seventy-three Days.**(a) Chilled Carcase.*

Pig 229 was fed 400 grams of chilled muscle. It remained healthy, but on subsequent testing for immunity to swine fever it showed considerable resistance, and was finally destroyed as it developed the chronic form of the disease.

(b) Salted Carcase.

Pig 299 was fed 400 grams of salted muscle. It developed typical symptoms of swine fever, viz., high temperature, swaying gait, prostration and bluish discolouration of the ears. These symptoms persisted for about ten days and then slowly disappeared, the animal remaining stunted and unthrifty in appearance.

A control pig, 289, placed in contact with 299, became infected and exhibited the cerebral symptoms which are not infrequently associated with swine fever in young pigs. This pig also made a partial recovery. A second healthy pig, 308, placed in contact with 299 and 289, developed typical symptoms of swine fever but it also made a partial recovery.

These three pigs were destroyed on the forty-second day of the experiment.

Results of Post-mortem Examinations.

Pig 299.—The lymphatic glands were much enlarged and showed on section petechial hæmorrhages. There were a few healing necrotic lesions situated near the ileo-caecal valve and two necrotic lesions in the larynx.

Pig 289.—All the lymphatic glands were enlarged and showed on section a marbled appearance. There was one large ulcer on the tongue and a few small ulcers inside the lips.

Pig 308.—Enlargement of the lymphatic glands was the only lesion present.

These post-mortem examinations were made in the presence of the late Mr. A. Holman Berry, F.R.C.V.S., who had a wide experience of swine fever, and in his opinion the lesions were similar to those which are usually associated with the chronic form of the disease.

As the portion of muscle fed was from an infected carcase, and as two control pigs contracted the disease by contact, it appears reasonable to conclude that some virus still persisted in the carcase although it had been in storage for seventy-three days.

Some of the pigs used about this period showed considerable resistance to swine fever, and as they had been purchased in the open market near London it is possible that they may have been obtained from districts in which swill-feeding is practised and where opportunities exist for pigs to develop some resistance to the disease from the ingestion of sub-infective doses of virus in material from infected carcasses. In view of the inconclusive results obtained in the muscle experiment, it was decided to carry out further work with this material and also with skin.

EXPERIMENT III.

Viability of the Virus in Muscle and Skin.

Pigs 346 and 347 were infected by feeding a portion of mixed spleens from two naturally infected pigs. They developed typical symptoms of swine fever and were shot and bled at the height of the temperature reaction. Portions of skin and muscle were preserved by chilling and salting in a similar manner to that described for the bone marrow experiment.

Control to Infectivity of the Muscle and Skin.

Muscle.—Pig 350 was fed 202 grams of a mixed sample of muscle, collected at time of death, from pigs 346 and 347.

This pig developed a slowly progressive emaciation and a profuse watery diarrhoea.

It was killed on the thirty-second day, and post-mortem examination showed enlargement of the lymphatic glands and numerous petechial hæmorrhages and ulcers in the large intestine.

Skin.—Pig 353 was fed 202 grams of a mixed sample of skin, collected at time of death, from pigs 346 and 347.

The pig showed a rise of temperature (105° – 106°) which started on the fifth day, lasted about a week and was accompanied by diarrhoea, but it eventually made a complete recovery.

It was killed on the twenty-fourth day and no lesions were found on examination.

Test I:—After Seventeen Days.

Muscle.

(a) *Chilled Carcase.*—Pig 349 was fed 202 grams of mixed muscle from pigs 346 and 347. It showed a small rise of temperature which lasted for four days, severe diarrhoea and considerable loss of condition, but eventually made a complete recovery.

It was killed for examination on the thirtieth day and the only abnormality present was marked enlargement of the lymphatic glands.

Pig 356, a control in contact with 349, developed the characteristic symptoms of swine fever and died on the twenty-first day. Post-mortem examination showed typical lesions of acute swine fever.

(b) *Salted Carcase.*—Pig 357 was fed 203 grams of mixed muscle from pigs 346 and 347. This pig stopped feeding for some days and was affected with diarrhoea, but eventually recovered. It was killed on the thirtieth day and no lesions were found on examination.

Pig 358, a control placed in contact with 357 developed profuse watery diarrhoea, but showed no rise of temperature. It became gradually weaker and was destroyed *in extremis* on the twenty-second day of contact.

Post-mortem examination showed enlargement of the lymphatic glands and considerable inflammation of the large intestine.

This pig was probably infected with swine fever, but the lesions found on post-mortem examination were insufficient to allow of a definite diagnosis.

Skin.

Pig 345 was fed 202 grams of skin from pigs 346 and 347.

It developed a slowly progressive type of disease, with a small rise of temperature and slight diarrhoea. It became gradually weaker and died on the forty-sixth day.

Post-mortem Examination.—All the lymphatic glands were enlarged, oedematous, and hæmorrhagic. The large intestines were inflamed and "button" ulcers were present near the ileocaecal valve.

Pig 366, a control in contact with 345, died of acute swine fever on the eighteenth day of contact.

It would appear reasonable to conclude from this experiment that the muscle and skin contained minute quantities of virus, insufficient to cause the death of the pigs to which it was fed, but sufficient after passage through them to set up fatal infection in the in-contact pigs. Instances of this type of infection are frequently met with under field conditions.

In this connection Birch says :—

"It has been shown that the carcasses of hogs that show no symptoms other than slight elevation of temperature and no lesions whatever, may contain hog-cholera virus sufficient to infect other hogs."

McBryde has shown that the minimal lethal dose of swine fever virus lies between the $1/300,000$ and $1/400,000$ of a cubic centimetre.

Conclusion.—The virus survived seventeen days in the skin and muscle of pigs killed before the appearance of clinical symptoms.

In view of the minute amount of virus present in the skin and muscle obtained from pigs 346 and 347 this experiment was discontinued.

SUMMARY.

EXPERIMENT III.—MUSCLE AND SKIN.

No. of Pig.	Material.	Method of Infection.	Method of Storage.	In Storage Days.	Result.
229	Muscle	By feeding	Chilled	73	Remained healthy
229 } 289 } 308 }	Muscle — —	By feeding Control-contact Control-contact	Dry salted — —	73 — —	Ill, recovered Ill, recovered Ill, recovered
349 } 356 } 357 } 358 }	Muscle — Muscle —	By feeding Control-contact By feeding Control-contact	Chilled — Dry salted —	17 — 17 —	Ill, recovered Died 21st day Ill, recovered Died 22nd day
345 } 366 }	Skin —	By feeding Control-contact	Dry salted —	17 —	Died 46th day Died 18th day

EXPERIMENT IV.

The spleens of three naturally infected pigs were ground up together and 100 grams fed to each of two pigs, 435 and 438.

These pigs developed acute swine fever and were shot and bled on the seventh day *when both were visibly ill*.

Portions of skin and muscle were dry salted and placed in cold storage as in the previous experiments.

*Test I.—After Twenty-three Days.**Salted Carcase.*

Muscle.—Pig 442 was fed 250 grams of muscle from pigs 435 and 438. It died on tenth day of acute swine fever.

Pig 440, a control in-contact with 442, died on the seventeenth day of acute swine fever.

Skin.—Pig 444 was fed 250 grams of skin from pigs Nos. 435 and 438. It died on the ninth day of acute swine fever.

Pig 439, a control in-contact with 444, died on the seventeenth day of acute swine fever.

Test II.—After Forty-two Days.

Muscle.—Pig 455 was fed 240 grams of muscle from pigs 435 and 438. It developed acute swine fever and was killed *in extremis* on the twenty-third day.

Pig 457, a control in-contact with 455, died of swine fever on the thirty-seventh day.

Skin.—Pig 454 was fed 240 grams of skin from pigs 435 and 438. It died of swine fever on the twenty-first day.

Pig 458, a control in-contact with 454, died of swine fever on the twenty-third day.

Conclusion.—Swine fever virus survived for forty-two days in the skin and muscle of salted carcasses.

Test III.—After Seventy-six Days.

Muscle.—Pig 453 was fed 240 grams of muscle. It remained healthy.

Pig 456, a control in-contact with 453, remained healthy.

Skin.—Pig 452 was fed 240 grams of skin. This pig developed chronic diarrhoea and became stunted and unthrifty.

Pig 450, a control in-contact with 452, remained healthy.

These four pigs were tested for immunity by being placed in contact with naturally infected animals and although all became ill none died.

It would appear that a minute quantity of virus still survived in the skin and muscle, insufficient to cause serious illness in the animals to which it was fed, but sufficient to confer a considerable degree of protection.

SUMMARY.

EXPERIMENT IV.—MUSCLE AND SKIN.

No. Pig.	Material.	Method of Infection.	Method of Storage.	In Storage Days.	Result.
442 } 440 } 455 }	Muscle — Muscle	By feeding Control-contact By feeding	Dry salted — Dry salted	23 — 42	Died 10th day — Killed in <i>extremis</i> , 23rd day
457 } 453 }	— Muscle	Control-contact By feeding	— Dry salted	— 76	Died 37th day Remained healthy
456	—	Control-contact	—	—	Remained healthy
444 } 439 } 454 }	Skin — Skin	By feeding Control-contact By feeding	Dry salted — Dry salted	23 — 42	Died 9th day Died 17th day Died 21st day
458 } 452 } 450 }	— Skin —	Control-contact By feeding Control-contact	— Dry salted —	— 76 —	Died 23rd day Ill, recovered Remained healthy

THE EFFECT OF "SMOKING" ON THE VIABILITY OF SWINE FEVER VIRUS IN BACON CARCASSES.

Although it appeared improbable that the light degree of smoking to which bacon is subjected under "trade" conditions would have any marked effect on the viability of the virus, it was considered advisable, in view of the large quantity of smoked bacon consumed in this country, to investigate this phase of the problem.

Tests carried out with self-recording thermometers during smoking showed the highest temperature reached on the outside of a carcase to be 85° F., and in the deep muscular tissue to be 82° F.

The strain of virus which had been used in the previous experiments had unfortunately been allowed to die out, and considerable difficulty was encountered in procuring another suitable strain, or at least in inducing a suitable degree of infection in pigs.

A number of strains were received through the courtesy of the Ministry's Veterinary Inspectorate Staff, with the information that they had been obtained from virulent outbreaks, but the majority of these strains when inoculated into adult pigs at the laboratory caused

only slight to moderate reactions, and the infection induced was too mild for virus viability experiments. In several instances, the pigs of the first passage died from acute swine fever, but those of succeeding passages showed only a weak or moderate reaction.

The pigs used were alleged to have been obtained from farms known to have been free of swine fever during recent years, but a statement of this kind is clearly of little value, as the pigs may not have been bred on the farm, but been purchased originally in the open market.

While it is not possible to state definitely the cause of these mild reactions without probing deeper into the question and using a large number of experimental animals of known origin, a consideration of the facts points to the pigs employed being of low susceptibility, presumably the result of either inherited or acquired immunity.

It would appear from this experience that, should an organised investigation of pig diseases be undertaken, it would be necessary to breed, under conditions which would exclude extraneous infection, a considerable proportion of the pigs required for experimental work.

EXPERIMENT V.

The Viability of the Virus in "Smoked" Carcasses.

Pigs 664 and 665 were infected with swine fever by feeding a saline suspension of spleen obtained from a pig infected by contact at the laboratory. These pigs were slaughtered on the sixth day at the height of the temperature reaction and before the appearance of visible symptoms. The carcasses were dry-salted for four days at room temperature, then washed in hot water to remove the salt, and smoked under "trade" conditions by a firm of bacon curers in Smithfield Market.

Control to Infectivity of the Skin, Muscle and Bone Marrow before Salting and Smoking.

Pig 668 was fed 200 grams of mixed skin from pigs 664 and 665. It died on the thirteenth day.

Pig 669 was fed 200 grams of mixed muscle. Died on the twelfth day.

Pig 670 was fed two long bones and the contained marrow. Died on the twelfth day.

Test I.

Bacon Two Days after Salting and Smoking.

Pig 676 was fed 200 grams of mixed muscle. Died on the thirtieth day.

Pig 677 was fed two long bones and the contained marrow. Died on the ninth day.

Pig 678 was fed 200 grams of mixed skin. Died on the tenth day.

Test II.

Bacon Nineteen Days after Smoking.

Pig 682 was fed 200 grams of mixed muscle. It died on the eleventh day.

Pig 685, a control in-contact with 682, died on the twenty-ninth day.

Pig 681 was fed two long bones. It died on the ninth day.

Pig 684, a control in-contact with 681, died on the fifteenth day.

Pig 680 was fed 200 grams of mixed skin. It died on the twenty-sixth day.
Pig 683, a control in-contact with 680, died on the seventeenth day.

Test III.

Bacon Thirty-seven Days after Smoking.

Pig 689 was fed 200 grams of mixed muscle. It died on the twenty-fourth day.

Pig 688, a control in-contact with 689, became sick for a few days and recovered.

Pig 687 was fed two long bones. Died on the tenth day.

Pig 690, a control in-contact with 687, died on the eighteenth day.

Pig 686 was fed 200 grams of mixed skin. Remained healthy.

Pig 691, a control in-contact with 686, remained healthy.

Conclusion.—The virus survived for 37 days (not the end-point) in bone marrow and muscle, and for 19 days in skin, of smoked carcasses.

SUMMARY.

EXPERIMENT V.

No. of Pig.	Material.	Method of Infection.	Method of Curing.	Days after Curing.	Result.
677	Bone Marrow	By feeding	Smoked	2	Died 9th day
676	Muscle	By feeding	Smoked	2	Died 30th day
678	Skin	By feeding	Smoked	2	Died 10th day
681	Bone Marrow	By feeding	Smoked	19	Died 9th day
684	Bone Marrow	Contact	Smoked	—	Died 15th day
682	Muscle	By feeding	Smoked	19	Died 11th day
685	Muscle	Contact	—	—	Died 29th day
680	Skin	By feeding	Smoked	19	Died 26th day
683	Skin	Contact	—	—	Died 17th day
687	Bone Marrow	By feeding	Smoked	37	Died 10th day
690	Bone Marrow	Contact	—	—	Died 18th day
689	Muscle	By feeding	Smoked	37	Died 24th day
688	Muscle	Contact	—	—	Ill, recovered
686	Skin	By feeding	Smoked	37	Remained healthy
691	Skin	Contact	—	—	Remained healthy

DISCUSSION.

The experiments here described prove the importance of bone marrow, muscle and skin from infected pig carcasses in the dissemination of swine fever. They confirm and extend the observations of Birch.

The great source of infection is clearly the bone marrow of pigs killed in the early stage of the disease, before the appearance of visible symptoms, because in it the virus is able to survive at least 73 days in sufficient concentration to set up infection when fed to susceptible animals.

The virus content of skin and muscle of pigs killed in the early stage of infection is relatively low, but as the disease progresses it becomes markedly increased, as shown by the acute infection induced by feeding material obtained from pigs exhibiting clinical symptoms.

The smoking of carcasses appeared to have no appreciable effect on the viability of the virus.

In view of recent knowledge obtained on the viability of the viruses of foot-and-mouth disease, fowl plague, Newcastle disease, and swine fever in the tissues of preserved carcasses, it is necessary to modify somewhat our ideas, and in future to regard the preserved carcase as the most dangerous channel through which these infections may be introduced.

CONCLUSIONS.

(1) The virus of swine fever survived 73 days (not end-point) in the bone marrow of "salted" and "chilled" carcasses.

(2) The virus survived 17 days in the skin and muscle of pigs killed before the appearance of clinical symptoms. There was some evidence that virus was still present in salted muscle after 73 days.

(3) The virus survived 42 days in salted skin and muscle of pigs killed *after* the appearance of clinical symptoms.

(4) The virus survived for 37 days (not end-point) in bone marrow and muscle, and for 19 days in skin, of smoked carcasses.

REFERENCES.

- Birch. 1917. *Amer. Vet. J.*, **51**, p. 301.
 Detmers. 1931. Cited in Dept. of Agricul. Rep. New South Wales.
 Henry, Seddon and Blumer. 1931. Dept. Agricul. Rep. New South Wales.
 Holth. 1927. *Norsk. Vet. Tidskr.*, p. 1. Cited by Zeller and Beller.
 McBryde. 1930. *J. Amer. Vet. Med. Ass.*, **29**, p. 155.
 McGilvray. 1912. *Amer. Vet. Rev.*, **41**, p. 444.
 Magnusson. 1927. *Svensk. Veterin. Tidskr. Jg. 32 H.3*, pp. 65-76.
 Minett. 1927. Second Progress Rep. Foot and Mouth Disease Res. Committee, London.
 Purchase. June, 1931. *Vet. Rec.*, p. 2.
 Rutherford. 1910-11. Vet. Director Rep. Canada.
 Zeller and Beller. 1929. *Cent. Bakt. Orig.*, **114**, p. 300.

I am much indebted to Colonel Dunlop Young, O.B.E., Chief Veterinary Inspector, Smithfield, for arranging with a firm of bacon curers to have the carcasses smoked.

THE ABSENCE OF SEROLOGICAL RELATIONSHIP BETWEEN BRUCELLA AND PASTEURELLA ORGANISMS.

By F. W. PRIESTLEY,

*Research Institute in Animal Pathology, Royal Veterinary College,
London.*

MALLMAN (1930) and Emmel and Boevers (1932) have recently published results which suggest that there is a serological relationship between the genera *Pasteurella* and *Brucella*.

Mallman met with a bovine serum which agglutinated bovine and porcine types of *Br. abortus* and *Pasteurella* organisms from various animal species; this serum also agglutinated *Pf. mallei* to a somewhat lower titre. He also found that, using immune sera prepared in rabbits, *Brucella* organisms were agglutinated by *Pasteurella* sera, and *Pasteurella* organisms were agglutinated by *Brucella* sera. For this reason he concluded that agglutination of *Br. abortus* by a suspected serum was not conclusive evidence of infection with that organism.

Emmel and Boevers, working solely with sera from fowls, obtained similar cross-agglutination reactions. With the sera from a number of uninoculated hens the results were completely negative, so that double infection as an explanation of their results can be excluded.

In the work recorded here an endeavour was made, without success, to confirm these results.

Suspensions for the tests were usually prepared by washing off the growth from agar plates with carbol-saline, filtering through paper and standardising to an opacity approximately equal to 2,000 by 10^6 *Bact. coli* per c.c.; with the *Pasteurella* organisms in a few cases meat infusion broth cultures, incubated for one to three days, were used. Twenty *Brucella* strains were tested, namely five *Br. abortus bovis* strains isolated by Sheather (1923), eleven *Br. abortus bovis* strains isolated at this Institute since 1929, and four *Br. abortus suis* strains received from Dr. I. F. Huddleson. These strains all agglutinated to approximately the same titre when tested against a *Brucella* serum. Forty *Pasteurella* strains were used, representative of *Pasteurellae* from most animal species which are commonly infected with these organisms. Twenty of these strains were received from the National Collection of Type Cultures, and twenty were isolated during the past year by workers in different parts of the world. Thirty of them gave the typical biochemical reactions of the genus, three were atypical in that they failed to produce indol, and seven were atypical in that they did not ferment mannite.

The sera used were mostly prepared in rabbits or hens by intravenous inoculation of pure cultures of various strains; one composite sample consisting of sera from a number of bovines naturally infected with *Br. abortus* was also used. The titres of the various sera for the homologous organisms ranged from 1 : 320 to 1 : 5120.

The agglutination tests were carried out by mixing 0.5 c.c. serum dilutions with 0.5 c.c. bacterial suspension in small tubes, which were then generally incubated in a water-bath at 56° C. for six hours and allowed to stand at room temperature for 30 to 60 minutes before a reading was recorded. A second reading was occasionally taken again after the tubes had been standing overnight, but in no case was there any material alteration in the result.

In the first experiment 20 *Brucella* suspensions were tested against 11 *Pasteurella* sera prepared in rabbits, the final dilutions ranging from 1:10 to 1:320. As no agglutination was observed in any case, 14 of the suspensions were retested against 7 of the sera in a dilution of 1:2, *i.e.* equal volumes of suspension and undiluted serum. Again no trace of agglutination was observed. A general summary of these results is given in Table I.

TABLE I.
AGGLUTINABILITY OF BRUCELLA SUSPENSIONS IN BRUCELLA AND
PASTEURELLA SERA.

Brucella sups.	Pasteurella Sera.					Brucella Serum (1)
	<i>aviseptica</i> (4)	<i>boviseptica</i> (2)	<i>lepiseptica</i> (2)	<i>suisetica</i> (2)	<i>muriseptica</i> (1)	
<i>Br. bovis</i> (16)	—	—	—	—	—	+
<i>Br. suis</i> (4)	—	—	—	—	—	+

The figures in brackets indicate the number of strains and sera tested.

In a similar manner 12 *Past. aviseptica* suspensions were tested against 3 *Brucella* antisera prepared in rabbits and against the composite bovine serum referred to previously. No trace of agglutination was observed although all these suspensions were agglutinated in some degree by a *Past. aviseptica* serum.

Similar completely negative results were obtained when 6 *Past. boviseptica*, 2 *Past. suisetica*, 6 *Past. muriseptica*, 9 *Past. lepiseptica* and 5 miscellaneous *Pasteurella* strains were tested against *Brucella* sera.

These results are summarised in Table II.

TABLE II.
AGGLUTINABILITY OF PASTEURELLA SUSPENSIONS IN BRUCELLA AND
PASTEURELLA SERA.

Pasteurella Suspensions.	Brucella Sera.			Past. serum (1)
	<i>abortus bovis</i> (1)	<i>abortus suis</i> (2)	Bovine serum (1)	
<i>Past. aviseptica</i> (12)	—	—	—	+
„ <i>boviseptica</i> (6)	—	—	/	/
„ <i>suisetica</i> (2)	—	—	/	/
„ <i>muriseptica</i> (6)	—	—	/	/
„ <i>lepiseptica</i> (9)	—	—	/	/
Miscellaneous <i>Past.</i> (5)	/	—	/	/
/ = not tested.				

These results were to some extent confirmed by the finding that absorption of Brucella sera by heavy suspensions of rough, blue, and fluorescent types of *Past. aviseptica* strains (Hughes, 1930) failed to lower the titre for *Br. abortus*. In connection with this point, however, it is recognised that absorption might leave one type of specific antibody untouched.

At this stage it was considered advisable to determine whether cross-agglutination would occur if hen sera were used in place of rabbit and bovine sera, since the results of Emmel and Boevers were based on the use of hen sera. Accordingly, two White Leghorn hens were immunised with *Br. abortus* (one with the *bovis* and one with the *suis* variety) and two with strains of *Past. aviseptica*. As was observed by Emmel and Boevers and by others, the hens inoculated with *Br. abortus* responded more rapidly and to a higher titre than those inoculated with *Past. aviseptica*.

The two Brucella sera prepared in hens agglutinated their homologous antigens to titres of 1 : 160 and 1 : 640 respectively, but failed to cause any agglutination of 12 *Past. aviseptica* suspensions in dilutions ranging from 1 : 10 to 1 : 320. Similarly, the two Pasteurella sera prepared in hens, while agglutinating their homologous antigens to titres of 1 : 128, failed to have any action on 10 Brucella strains.

These results are shown in Table III.

TABLE III.

AGGLUTINABILITY OF BRUCELLA AND PASTEURELLA SUSPENSIONS IN HEN SERA.

Suspensions.	Hen Sera.	
	Brucella (2)	Pasteurella (2)
Brucella (10)	+	—
Pasteurella (12)	—	+

It is perhaps worth while noting that the two Pasteurella sera gave a heavy flocculent precipitate in dilutions up to 1 : 8, while the Brucella sera did not show this. This precipitate was presumably the non-specific one not uncommonly met with in hen serum, since it appeared quite as readily when the serum was mixed with saline as when mixed with suspension.

As a final effort, a number of cross-agglutination tests in which rabbit and hen sera were used were carried out in the dry incubator at 37° C., readings being recorded after 24 hours. Again all sera agglutinated the homologous genus, but had no effect upon the heterologous genus.

DISCUSSION.

The results recorded are in striking contrast to those of Mallman and of Emmel and Boevers. It may be stated quite definitely that,

with the technique used here, there is no cross-agglutination between *Pasteurella* and *Brucella*.

The agglutinability of *Pasteurella* organisms is notoriously erratic ; a strain may be agglutinable one day and completely inagglutinable a week later with the same sample of serum. The underlying causes of this are obscure and are now being studied, but at present it must be realised that failure of two strains of this genus to flocculate in a serum agglutinating one of them does not necessarily mean lack of antigenic similarity. However, in view of the fact that similar negative results were obtained with 40 *Pasteurella* strains, a proportion of which at any rate were agglutinated by *Pasteurella* sera, the conclusion that *Pasteurella* organisms are inagglutinable with *Brucella* sera seems to be clearly justified.

Brucella suspensions do not behave in the same erratic manner, and consequently the results obtained when these were tested against *Pasteurella* sera are of special significance in supporting the general conclusion.

Unfortunately, neither Mallman nor Emmel and Boevers give details of their technique, and it is therefore possible that certain differences in this respect may explain their rather curious results. In any case it can be definitely stated that provided agglutination tests are performed in accordance with the technique outlined above no confusion in the matter of diagnosis between *Pasteurella* and *Brucella* disease need arise.

CONCLUSIONS.

1. Under the conditions outlined, *Brucella* organisms are not agglutinated by *Pasteurella* sera.
2. Under similar conditions *Pasteurella* organisms are not agglutinated by *Brucella* sera.
3. There is no serological relationship between the genera *Brucella* and *Pasteurella*.

BRUCELLA ABORTUS IN THE HORSE.

By HUGH MACKENZIE DUFF,

*Veterinary Laboratory, Ministry of Agriculture and Fisheries,
Weybridge.*

SEVERAL instances have been recorded in America and on the Continent of the isolation of *Br. abortus* from lesions in the horse, but up to the present time no case seems to have been reported of the isolation of this organism from horses in Great Britain. The object of the present paper is to record the recovery of *Br. abortus* from a withers abscess in a horse near Windsor.

It has been known for a number of years that poll evil and fistulous withers are conditions more commonly found in agricultural horses than in horses used continuously in towns (Davis 1922), but, so far, no satisfactory explanation appears to have been put forward to account for this peculiar distribution.

Many factors have been considered from the point of view of etiology. Trauma and pyogenic bacteria have been most frequently implicated; in addition, studies of abnormalities of the ligamentum nuchae of the horse (Bovett 1861, Railliet and Henry 1910, Robson 1918, Van Volkenburg 1920, Caslick 1921, Ackert and O'Neal 1930, Pillers 1931) have shown that a small worm belonging to the Filariidae is very often associated with these conditions. No statistics appear to be available, however, to indicate with what frequency this parasite is to be found in apparently normal horses. Bacteriological examinations of the fluid from the abscesses have demonstrated that *Br. abortus* alone or accompanied by other organisms is not uncommonly connected with such lesions.

In tests carried out in Germany during the war (Fontaine and Lutje 1919) with *Br. abortus* on the sera of 3,419 horses, it was noted that out of the 17 horses which reacted positively to the agglutination test 12 were affected with fistulous withers. *Br. abortus* was isolated from 4 of these cases. In a report to the French Veterinary Academy on localised pyogenic infections in horses (Rinjard and Hilger 1928) details are given of studies of 15 cases of poll evil and fistulous withers. The blood sera of 12 of the 15 animals gave positive agglutination results. The organism was isolated from abscesses in two horses.

The possibility of the association of *Br. abortus* with abortion in mares was demonstrated in America (McNutt and Murray 1924) by the isolation of the organism from an aborted equine foetus. As a result of this discovery work was undertaken at Minnesota, America, on *Br. abortus* infection in horses (Fitch, Delez and Boyd 1930). Of 46 horses suffering from poll evil and fistulous withers which were

placed under observation, 40 gave positive reactions to the agglutination test. *Br. abortus* was recovered from samples of pus from 3 out of 24 horses. In later studies at the same laboratory (Fitch, Bishop and Boyd 1932) agglutinins were found to be present in 76 per cent. of the horses under test. The organism was isolated from 22 per cent. of the specimens of pus examined. Five attempts were made to infect horses experimentally. In one case a condition indistinguishable from poll evil was set up by the injection of a suspension of *Br. abortus* between the portions of the ligamentum nuchae at the poll. The blood serum of the horse agglutinated up to 1 in 2,000 two months after inoculation. The organism was recovered from the lesion.

Work carried out in Holland (Van der Hoeden 1930) with specimens of blood from 424 horses showed that agglutinins were present in the sera of 130. *Br. abortus* was obtained from 8 of 12 samples of fluid from fistulous withers in reacting horses. Further tests (Van der Hoeden 1931) revealed that 14 out of 15 horses affected with neck and throat abscesses and 2 out of 6 horses with abscesses of the sternum gave positive agglutination and complement fixation reactions with *Br. abortus*. Efforts to infect horses artificially (Van der Hoeden 1932) failed to produce typical lesions. The same worker (Van der Hoeden 1932) found on the examination of the sera collected from 482 horses at slaughterhouses that 72 of the samples agglutinated up to 1 in 100 with *Br. abortus*. In addition, in observations on 67 horses suffering from bursitis, arthritis, and abscesses in the region of the neck and shoulders, he noted that 52 gave positive agglutination results with titres of not less than 1 in 400, some reacting as high as 1 in 12,800. *Brucella* strains were isolated from 30 out of 48 specimens of pus from such abscesses.

Statistics of *Br. abortus* infection in horses in Russia (Makkawejsky *et al.* 1931) reveal that of 375 horses, closely associated with infected cattle in 14 agricultural districts, 75 reacted to the agglutination test in a titre of 1 in 100 or over. Among the reacting animals were three mares which had previously aborted, and several horses suffering from swellings on the poll and withers and from enlargements of the knee and fetlock joints. The isolation of *Br. abortus* is not mentioned in the report on this work.

The association of *Br. abortus* with poll evil and fistulous withers has also been recorded as a result of observations in Australia (Bennetts and Filmer 1931). Agglutination titres up to 1 in 2,000 were noted. No attempt was made to cultivate *Br. abortus* from the lesions.

Recent investigations in Sweden (Hulten 1931, Magnusson 1932) have definitely established that *Br. abortus* is associated with some abscesses on the poll and withers in horses in that country.

During the past year details have been published (Schoop 1932) of further observations in Germany. Nine horses were brought under investigation, of which six suffered from fistulous withers and three from poll evil. Six animals reacted to the agglutination test

in a titre of 1 in 200. The organism was recovered from three of the former and all of the latter conditions. It was found in East Prussia (Hieronymi 1932) that the sera of ten of a total of twelve horses affected with poll evil and fistulous withers possessed agglutinating and complement fixing properties in positive amounts.

Recently an opportunity occurred, through the courtesy of Captain Walker of Slough, to examine a case of suspected *Br. abortus* infection in a horse in the vicinity of this laboratory (Weybridge).

The animal, an aged, grey cross-bred Suffolk mare, was observed by the owner on November 2nd to have swellings on the poll and withers. These gradually increased in size and became very tender. On November 16th the abscesses on the poll burst on each side of the mane.

The mare was examined on November 23rd, when it was found that the abscess on the withers was "pointing" towards the off side. About 80 c.c. of yellowish, glutinous, blood-tinged fluid was aspirated from the lesion with aseptic precautions. A specimen of blood from the jugular vein was also obtained.

It is of interest that this animal is usually pastured on common land on which abortions in cattle are reported to have frequently occurred. The cows of the same owner do not graze on the common, and according to the owner's statement they are free from contagious abortion.

Various types of culture media, including liver infusion agar, were inoculated with fluid from the abscess and incubated at 37° C. in an atmosphere of 10 per cent. CO₂. Another series of culture tubes containing the same types of media were inoculated and incubated aerobically at 37° C.

In 72 hours numerous small, dewdrop-like colonies developed in the culture tubes containing liver infusion agar which had been incubated in the CO₂ jar. Upon microscopic examination, all of these colonies were found to consist of the same type of organism, *i.e.*, a small, pleomorphic, Gram-negative, non-motile, non-capsulated cocco-bacillus, which showed a tendency towards granular staining.

Neither acid nor gas was produced in glucose, lactose, dulcitol, maltose, mannite, saccharose, sorbitol, inulin, laevulose, raffinose, arabinose, rhamnose, adonite, galactose, zylose, dextrine, inositol, or salicine. No indol was produced. Litmus milk showed slight alkalinity in four days and distinct alkalinity in ten days. Potato cultures possessed the characteristic yellowish-brown coloration.

Growth was very slow in the culture tubes incubated aerobically, but sub-cultures from these grew well under ordinary atmospheric conditions. After a few transfers the growth on liver infusion agar became rather coarse and built-up in appearance.

The blood serum and pus fluid from the mare agglutinated up to 1 in 500 with five different antigens prepared from strains of *Br. abortus* isolated from bovine foetuses from various parts of this country.

The sera of guinea-pigs inoculated with 1 c.c. of a suspension of culture growth agglutinated with different antigens up to 1 in 50 at the seventh day and up to 1 in 100 at the fourteenth day after inoculation. As a matter of interest, cultures were made from the blood drawn from the guinea-pigs by heart puncture for the agglutination test. In each case the organism was recovered in pure culture. Positive culture growths were obtained from typical spleen lesions in guinea-pigs five weeks after the inoculation of pus. Fermentation tests, potato cultures, and smears confirmed the observations previously made with the organism isolated directly from the abscess fluid.

On carrying out tests with antigens prepared from this organism it was found that agglutination occurred spontaneously in the presence of salt solutions.

Dilutions of known positive and negative sera were made containing 0.05, 0.1, 0.2, 0.425, 0.85, 1.4, 2.8, and 12 per cent. salt and tested against preserved and unpreserved antigens prepared from growth on ordinary agar and on liver infusion agar. In each case a marked tendency to spontaneous agglutinability was observed.

A method of extraction by alcohol and chloroform (White 1928) was tried with success. Using a suspension of extracted organisms, agglutination tests were carried out with known positive and negative sera in dilutions containing 0.1, 0.4, 0.85, 2.8, 6 and 12 per cent. sodium chloride. It was found that the antigen thus prepared was reliable in concentrations of 0.4, 2.8, 6 and 12 per cent. NaCl but rather unstable in 0.1 and 0.85 per cent. A suspension of extracted organisms prepared with 12 per cent. saline agreed with five *Br. abortus* antigens when used in agglutination tests against numerous positive and negative sera.

In recording this instance of the isolation of *Br. abortus* from an English horse, it is not now proposed to discuss the significance of the presence of this organism in abscesses of the poll and withers in horses. Further work is being undertaken, and it is hoped, as a result of the information obtained, that it may be possible at a later date to express an opinion on the probable relationship of *Br. abortus* to the etiology of suppurative conditions in horses.

SUMMARY.

1. An organism isolated in pure culture from a withers abscess in a horse proved to be indistinguishable microscopically, culturally and serologically from *Br. abortus*.

2. The blood serum and abscess fluid from the horse under observation agglutinated various *Br. abortus* antigens up to, and including, 1 in 500. The same titre was observed when a suspension of the organism from the abscess was used.

3. The coarse character of the culture growth and the marked tendency to agglutinate spontaneously in the presence of salt solutions seem to indicate that the organism is a rough strain of *Br. abortus*.

REFERENCES.

- Ackert, J. E., and O'Neal, W. S. 1930. *J.A.V.M.A.*, Vol. lxxvii, p. 28.
- Bennetts, H. W., and Filmer, J. F. 1931. *Australian Vet. J.*, Vol. vii, p. 74.
- Bovett, E. J. 1861. *Veterinarian*, p. 515.
- Caslick, E. A. 1921-22. *Rept. to New York Vet. St. Coll.*, p. 162.
- Davis, W. R. 1922. *Vet. Rec.*, Vol. xii, p. 226.
- Fitch, C. P., Delez, A. L., and Boyd, W. L. 1930. *J.A.V.M.A.*, Vol. lxxvi, p. 17.
- Fitch, C. P., Bishop, L., and Boyd, W. L. 1932. *J.A.V.M.A.*, Vol. lxxx, p. 69.
- Fontaine and Lutje. 1919. *Zeitschr. f. veterinarkunde*, Vol. xxxi, p. 15.
- Hieronymi, E. 1932. *D.T.W.*, Vol. xl, p. 593.
- Hulten, O. 1931. *Svensk Vet.-Tidskr.*, Vol. xxxvi, p. 25.
- McNutt, S. H., and Murray, C. 1924. *J.A.V.M.A.*, Vol. lxxv, p. 215.
- Magnusson, H. 1932. *Skand. Vet.-Tidskr.*, Vol. xxii, p. 95.
- Makkawesky, W. N. *et al.* 1931. *D.T.W.*, Vol. xxxix, p. 86.
- Pillers, A. W. N. 1931. *Vet. Rec.*, Vol. xi, p. 1246.
- Railliet and Henry. 1910. *Comp. Rend. Soc. Biol.*, Vol. lxxviii, p. 248.
- Rinjard, P., and Hilger, B. A. 1928. *Bull. Acad. Vet. de France*, Vol. i, p. 272.
- Robson, J. 1918. *Vet. Rec.*, Vol. xxx, p. 348.
- Schoop, G. 1932. *D.T.W.*, Vol. xl, p. 520.
- Van der Hoeden. 1930. *Tijdschr. Diergen*, Vol. lvii, p. 15.
- Van der Hoeden. 1931. *Tijdschr. Diergen.*, Vol. lviii, p. 1321.
- Van der Hoeden. 1932. *Zeitschr. Infektr.*, Vol. xlii, p. 1.
- . 1932. *Tijdschr. Diergen.*, Vol. lix, p. 612.
- Van Volkenburg, H. L. 1920-21. *Rpt. New York St. Vet. Coll.*, p. 181.
- White, P. B. 1928. *J. of Path. and Bact.*, Vol. xxxi, p. 423.

CHLORAL HYDRATE AS A GENERAL ANÆSTHETIC FOR THE FOWL.

By NORMAN HOLE, B.SC., M.R.C.V.S.

Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge.

THE anæsthetisation of poultry is a subject of practical importance to only a few research workers who occasionally find it necessary to perform inoculations or operations of such a nature that humanity, or the law, demands insensibility to pain. Very little information on the subject is obtainable; Pearl, Surface and Curtis (1) advise the inhalation of ether following a subcutaneous injection of atropine sulphate; Kaupp (2) uses chloroform administered through a mask. Neither authority states how long the subject can be kept anæsthetised, but both note the susceptibility of the bird to either chloroform or ether, and that death quickly follows an overdose. This susceptibility is most probably due, as pointed out by Pearl, Surface and Curtis, to the peculiar respiratory system of the bird, which includes not only the lungs, but also air sacs and air cells extending even into bony tissue. The writer's experience is that chloroform, administered by inhalation, can only be used with safety to produce complete anæsthesia of up to 30 seconds duration; the susceptibility of different individuals varies considerably.

It seems evident, therefore, that inhalation is an unsuitable method to adopt for the fowl, and a few experiments, which are the subject of this article, have shown that chloral hydrate, injected directly into the blood stream, is a fairly safe anæsthetic which will produce a complete anæsthesia of over half-an-hour's duration.

Intravenous infusion anæsthesia is not a new idea. Bastedo (3) mentions that chloroform has been tried intravenously but abandoned as too dangerous; ether [Bastedo (3) and Cushney (4)] has been used with more success, but is dangerous owing to the liability to hæmoglobinuria. Cushney (4) describes the successful but risky use of hedonal in this manner, while Bastedo (3) mentions a mixture of paraldehyde and ether for use in minor operations. These references all relate to human practice.

Chloral hydrate is not usually regarded as a general anæsthetic; it is commonly used as a soporific administered by the mouth or per rectum. It can be made to produce general anæsthesia, however, by these routes, providing a large enough dose is used, but the amount necessary is usually sufficient to cause death. It seems, however, that the fowl offers considerable resistance to chloral hydrate poisoning when it is injected into the blood. Intravenous inoculation in the fowl is very simple, the most suitable vein lying on the underside of the wing close to its junction with the body.

Preliminary Experiments.

A solution of chloral hydrate was made up to contain 1 gm. of the solid in 10 c.c. of normal saline. Birds weighing between 51 oz. and 58 oz. were inoculated with from 1 c.c. to 4 c.c. of this solution. The smallest dose put the bird off its legs and made it sleepy; It was never completely anæsthetised, however, and recovered within the hour. Two to four c.c. completely anæsthetised; the birds were "under" within 30 secs. to 2 min., and remained, with no corneal reflex, for 18 min. to 1 hour. The effect of the drug on respiration varied considerably; small doses produced little obvious change, but with larger doses the breathing sometimes became irregular and gasping, and sometimes remained regular but very shallow.

Further Experiments.

It was considered necessary to obtain a rough estimate of the minimum lethal dose, and for this purpose normal saline solutions containing from 0.3 gm. to 0.8 gm. per c.c. were made up. Birds used were about the same weight and in good condition. Fowls receiving 0.6 gm. or more in 1 c.c. of saline always died within two minutes, with cyanosis of the comb. No fowl succumbed to 0.4 gm. or less, and in every case recovery was complete by the forty-eighth hour.

The Effect of Weight.

The weight of the bird did not appear materially to influence the toxicity of the chloral hydrate; the experiments performed were not sufficient to justify conclusions, but the lethal dose for 85 oz. birds seemed essentially the same as for 55 oz. birds.

The Effect of Diluting the Solution.

Again only a few such experiments were carried out, but it seemed that the fowls could tolerate larger amounts of chloral hydrate in dilute solution than they could in concentrated solution. The inoculation of 0.6 gm. in 1 c.c. always proved fatal; the same amount in 2 c.c. was tolerated, however, and of two birds receiving 0.8 gm. in 4 c.c. of saline one survived and the other did not die until 18 hours later.

The method of intravenous injection employed differed essentially from intravenous infusion in human beings in that only one final dose was given. The effect of repeated doses has not been tried.

CONCLUSIONS.

1.—Chloral hydrate has proved useful as a general anæsthetic for the fowl; the intravenous injection is simple, and there appear to be no after effects.

TABLE SHOWING CHLORAL HYDRATE INJECTIONS.

<i>Fowl. Weight.</i>	<i>Injection.</i>	<i>Effect.</i>	<i>24 hours later.</i>	<i>10 days later.</i>
1822	0.01 g. in 0.1 c.c.	Nil	Normal	Normal
1839	0.1 g. in 1 c.c.	Off its legs ; dazed 1 hr.	Normal	Normal
1824	0.2 g. in 2 c.c.	" Under " for 15 mins.	Normal	Normal
1870 51 oz.	0.2 g. in 2 c.c.	" Under " in 2 min. Lasting 29 mins.	Sluggish	Normal
1833 55 oz.	0.25 g. in 2.5 c.c.	Do. Lasting 18 mins.	Normal	Normal
1869 58 oz.	0.3 g. in 3 c.c.	Do. Lasting 23 mins.	Normal	Normal
1830 56 oz.	0.4 g. in 4 c.c.	" Under " 30 secs. Lasting 60 mins.	Bright. Off Legs	Normal
1836 55 oz.	0.4 g. in 1 c.c.	" Under " for 1½ hours	Listless	Normal
1837 55 oz.	0.4 g. in 1 c.c.	" Under " for 1½ hours	Listless	Normal
1832 54 oz.	0.6 g. in 1 c.c.	Cyanosis and Death 2 min.	—	—
1834 56 oz.	0.8 g. in 1 c.c.	Cyanosis and immediate death	—	—
590 76 oz.	0.3 g. in 1 c.c.	" Under " for 30 mins.	Normal	Normal
502 87 oz.	0.6 g. in 1 c.c.	Cyanosis and immediate death	—	—
937 84 oz.	0.6 g. in 1 c.c.	Do.	—	—
503 91 oz.	0.6 g. in 2 c.c.	" Under " for 40 mins.	Normal	Normal
566 84 oz.	0.6 g. in 2 c.c.	Temporary cyanosis. " Under " 1 hr.	Off legs Trembling	Normal
946 80 oz.	0.8 g. in 4 c.c.	Do.	Do.	Normal
959 92 oz.	0.8 g. in 4 c.c.	Anæsthesia. Died 18th hr.	—	—
Injection amounts in grammes per cubic. cent. of saline. Injections all intravenous route.				

REFERENCES.

- (1) Pearl, R., Surface, F. M., and Curtis, M. R. Diseases of Poultry. Macmillan Company, New York.
- (2) Kaupp, B. F. Poultry Diseases. American Veterinary Publishing Co., Chicago.
- (3) Bastedo, W. A. Materia Medica. Pharmacology and Therapeutics. W. B. Saunders and Co., Philadelphia and London.
- (4) Cushney, A. R. Pharmacology and Therapeutics. Churchill, London.

VACCINATION AGAINST CONTAGIOUS BOVINE ABORTION.

By SIR JOHN MCFADYEAN.

AT the annual meeting of the National Veterinary Medical Association in August last a paper contributed by Dr. Horner Andrews, on "Vaccination Against Bovine Contagious Abortion and the Relation of this Disease to Undulant Fever of Man," contained the following paragraphs:—

"The Ministry of Agriculture has issued a live vaccine since the time of the Departmental Committee, and the annual demand for it has varied only slightly, averaging 25,000 doses. From the nature of the problem it would be impracticable to attempt to obtain detailed reports of the results of inoculations, or to compile statistics of any value. In such matters favourable results are not normally reported, after the first year or two, but definitely unfavourable results may be expected to give rise to immediate complaint. For many years we have felt that the general results must be favourable, as the same practitioners obtained the vaccine year after year, and complaints of failure have always been very few indeed. Recently, the Ministry decided to impose certain additional restrictions on the issue and use of the vaccine, and we communicated with all the veterinary practitioners who have obtained vaccine from us in recent years. This resulted in the receipt of a large number of letters giving information concerning the results noted in practice, and their general tone was highly favourable to the vaccine. Only one practitioner condemned it, but he appeared to have used it only in one herd, and one may doubt whether the effects described by him should be attributed to the vaccine. The general testimony in favour of the vaccine includes some very interesting points. Uninoculated controls cannot be deliberately set aside in practice, but for various reasons animals have sometimes been missed, and have served as controls, although on a very small scale. Successful inoculation for a couple of years on grossly-infected premises has been suspended for one year with disastrous results that were again checked by vaccination. A few veterinarians have also found that after preventing abortions for a number of seasons with the use of live vaccine they have been able to stop inoculation altogether, the infection having apparently disappeared.

"Over a period of many years, definite failures of the vaccine have been reported in very few instances, but in those instances the failure has been almost complete. It has not been possible to investigate most of these cases at all fully, and in many of them the persistent abortions may well have had some cause other than *B. abortus*, but in a few instances the cause has undoubtedly been *B. abortus*. We have not been able to show that any special type of strain was present, and we have as yet no explanation for these very occasional failures.

"The available evidence appears to justify the belief that in a large proportion of cases the use of live cultures reduces the number of abortions to very small proportions, or even prevents their occurrence entirely. It appears, therefore, that the method is of very real economic value, and that

its use is fully justified unless in some way it causes definite injury. Three objections to the method have been put forward—that it causes sterility, that it encourages the spread of the disease, and that it increases the risk of human infections.”

The present author ventures to express the opinion that the facts summarised in the above extract do not prove, or even make it probable, that the system of vaccination encouraged by the Ministry of Agriculture has had any effect in reducing the number of infected animals in the herds in which it has been practised. It appears to him that if the vaccine was supplied to veterinary surgeons for uncontrolled use in their practices in the expectation that the value or the safety of the method could be determined in that way it was bound to end in disappointment.

The information supplied by the practitioners has been so lacking in precision that no statistics of any value could be compiled from it, and it is not unfair to conclude that those who made reports generally realised that they had no important facts to communicate. That is also what might be inferred from the discussions in regard to the results of vaccination that have been reported in recent years ; here again it might truthfully be said the “ general tone ” of the discussion has been favourable to the vaccination, but the speakers have nearly always exercised a remarkable restraint in the provision of facts as opposed to unsupported opinions. One is thus left wondering what facts in connection with the use of the vaccine were considered favourable.

The value of vaccination must be measured by the extent to which it reduces the number of abortions and prevents fresh infections in the herd. That method of measurement cannot be employed in general practice, and there is no other. The preceding two sentences state the crux of the question.

Dealing first with the effect of vaccination in preventing abortions in a herd, it is obvious that the evaluation of this effect demands an estimate of what would have been the incidence of abortion in the herd if the cows and heifers had not been vaccinated. In the conditions under which the vaccine supplied by the Ministry was used the practitioner cannot have had any basis of comparison except the number of abortions during a year or other period before vaccination, and there is immense experience to show that such a method cannot yield trustworthy conclusions. The number of cases of abortion that occur in different infected herds, or in the same herd, in successive years is so variable as to defy prediction or the formation of a firm idea as to what should be regarded as the normal or average rate of abortion in infected herds in which the disease is allowed to run what may be called its natural course.

With regard to the second point—the effect of vaccination in arresting the spread of infection in a herd, it is equally plain that the reports sent to the Ministry of Agriculture must have been devoid of useful information because clinical methods are quite unreliable for

the diagnosis of the disease in animals that do not abort. The practitioners cannot have had any idea regarding the number of infected animals in the herd either before or after the vaccination. So far as they knew, the number might have been doubled after vaccination.

Is there, it may now be asked, any conceivable method by which the value of vaccination for the control or prevention of the disease might be determined? The answer must be in the affirmative. Notwithstanding the difficulties in the way of obtaining conclusive evidence by the methods at present employed, results that might either justify or condemn the continuance of vaccination could be obtained by properly controlled large-scale experiments in infected herds, in which half, or approximately that proportion, of the animals would be vaccinated, the remainder being left unvaccinated to serve as so-called controls. In no country has this method been tried in a way that strictly complies with these conditions. When it is used in only a few herds, and only a small proportion or none of the animals are left as controls, the results cannot be expected to wipe out the uncertainties arising from the varying incidence of infection and the varying abortion rate in different herds in successive periods independently of vaccination.

From the fact that such experiments have not hitherto been made, it may be concluded that they have everywhere been found impracticable on account of the expense and the obvious difficulty in obtaining the consent of the owners of the herds. It must be acknowledged that such experiments minus the use of proper controls have sometimes yielded results in favour of vaccination, but before any reader decides to accept such evidence as conclusive he would do well to consider the account of the controlled experiments carried out in Denmark which he will find at page 62 in this number of the Journal.

The chief purpose of this article was to consider the existing evidence regarding the practical value of vaccination in the control or prevention of the disease, but it cannot be considered entirely out of place to refer to the question of its safety. It has actually been suggested that vaccination at least deserves the recommendation that "if it does no good it does no harm," but that is far from certain.

Incidentally it may be pointed out that although the word vaccination long ago lost its original meaning, its extension to include the use of living bacteria with all their natural virulence is open to objection inasmuch as it tends to lull suspicion of danger. In reality, the injection of living unattenuated cultures of the *B. abortus* into healthy cattle would be better designated "inoculation."

The evidence collected in this country is very far from proving that no harm has been caused by the inoculation of 25,000 cows and heifers annually during the last 20 years. Wide experience suggests that probably the majority of these animals were healthy at the time of vaccination, and, whatever may be the final result of vaccination, its immediate effect is to cause a 100 per cent. infection of the

herd. In unvaccinated herds a blood test seldom shows that half the animals are infected.

It is not suggested here that the risk of lasting infection from vaccination of non-pregnant cows is a great one, but it exists, and it is therefore impossible to affirm that vaccination is always harmless. It is very disturbing to learn that in some of the herds in this country the pregnant cows have also been inoculated with massive doses of live bacilli. It cannot be doubted that such a procedure involves a great risk of causing permanent or lasting infection of the udder, which clinical examination would not detect.

The fact that vaccination as hitherto practised has failed to produce evidence that it is capable of preventing the spread of the disease in an infected herd is not the only reason why its discontinuance should now be carefully considered. There are two further facts that point in the same direction, the first being that vaccination has no curative effect on animals already infected, which may form a considerable proportion of the herd. Advocates of vaccination appear generally to have assumed that the contrary is the case. It may also be pointed out that in 1914, when this vaccination was first introduced, it was not known that in many cases the animals remain diseased for several years. The second reason is that, at the time when the Ministry of Agriculture began to distribute the vaccine and encourage its use, it had not been realised that a large proportion of cows become "carriers" through infection of the udder, with consequent danger of infection to human beings.

It would perhaps be unfair to take no notice of one item of evidence in favour of the continuance of vaccination. Some of the largest users of the vaccine have said that the results at any rate satisfy their clients. It may be recalled that the keeping of a male goat along with the cows had the same justification.

It has also been put forward as a reason why the present system of vaccination should be continued that if it were discarded practitioners would not know what to advise when consulted about the disease. There does not appear to be any reason for such a despondent outlook. Organised research can be resumed on a better basis when this wrong trail has been abandoned, and meanwhile the veterinary practitioner is still able to give valuable advice if he is consulted in good time.

There is no cut-and-dried or stereotyped method of dealing with outbreaks of the disease, because of such varying conditions as the size of the herd, the value of the animals (milk or pedigree), and the facilities for separation or isolation. It is therefore only possible to give here some general indications with regard to what appear to be the best means in the majority of cases.

It is in cases of recent infection of herds that veterinary practitioners can offer advice of the greatest value to the owner, and an effort should be made to spread the knowledge of this fact among owners of breeding herds.

It may provisionally be assumed that infection is recent when one is informed that the present abortion is the first one for a number of years. What is to be advised in such a case? Certainly not that the owner should consent to have the entire herd forthwith inoculated with the bacilli of contagious abortion, whether alive or dead. If it has already been established by a blood test of the cow, or otherwise, that the case which has occurred is one of contagious abortion, directions should be given for such disinfection and isolation as is possible, and the owner should be advised to consent to a blood test of all the animals of breeding age in the herd. In some cases the test may indicate that only a very small number of the animals are infected, and in these the possibility of arresting the outbreak by disposing of the infected animals (by any means not involving concealment of the fact that they are infected) should be taken into consideration.

If, on the other hand, at the time when advice is sought the herd already contains a considerable number of animals that have aborted, or a blood test shows a large proportion of infected animals, the owner may be informed that there is no way by which the disease could be quickly eradicated. Experience has shown that the best method of dealing with such cases is as follows, provided it is not absolutely necessary to introduce a large number of new animals annually during the next few years.

The owner should be advised not to sell cows that have aborted, but to retain them in the herd unless there are other reasons for putting them out, and to allow them to be served after an interval of not less than three months. It is, of course, assumed that appropriate treatment in the way of disinfection of the genital passages will be carried out in all such cases. The owner should also endeavour to avoid the introduction of new animals for at least two years.

When this plan is adopted it is not necessary to effect a separation between the diseased and the healthy, though disinfection and temporary isolation should be practised when a cow aborts. Calves born in the herd may be reared, even from cows known to be infected, and be put with the bull at the usual age provided the blood test is negative. The weak point in this method is the ultimate necessity of introducing fresh animals into the herd, with consequent risk that they will become infected, but that may be diminished by keeping the pregnant heifers separate until they have calved. After a few years in which no abortions have occurred a blood test of all the animals of breeding age may show that only a few remain infected, and in that case the advisability of putting them out of the herd should be considered.

The plan here recommended was founded on experience and was often used with satisfactory results before the introduction of vaccination, and without the help which can now be obtained from the agglutination test. The success of the method depends on two facts: (1) that about 80 per cent. of cows do not abort a second time when kept on in the herd, and (2) that in the great majority of out-

breaks the number of cases of abortion soon tends to diminish provided what may be called the fire of infection is not fed by introducing fresh susceptible animals. In the words of the late Prof. B. Bang (1907), "The virulence of the disease appears to become attenuated."

The method of course is not applicable to dairy herds entirely composed of cows in milk, and annually recruited by the purchase of fresh cows, newly calved, in the market. It must further be admitted that it is not a method that can be expected to eradicate the disease from a herd immediately, but in that regard it is better than the one that blindly relies on vaccination.

Grandiose schemes for eradicating the disease are out of the question, and efforts should have regard to the fact that at least for many years to come regulations must have the approval and the assistance of the owners of infected herds. Success will largely depend on the knowledge and intelligence of the veterinary practitioners in agricultural districts throughout the country. They should be encouraged to keep detailed records of the facts in connection with every outbreak, and such records might be collected by the Ministry of Agriculture for critical study and eventual publication.

CONCLUSIONS.

1. The so-called vaccination against bovine contagious abortion is an intentional infection of cows and heifers with enormous doses of living, fully virulent, cultures of the organism that is the cause of the disease.

2. Notwithstanding the fact that it has been practised in hundreds of herds in this country during the past 20 years, there has not yet been published satisfactory evidence that it is capable of preventing the spread of the disease or reducing the number of cases of abortion in infected herds.

3. The opinions of the practitioners who have used the vaccine are deprived of value by the fact that they did not know the extent to which the herds were infected either before or after the vaccination.

4. The inoculation of pregnant cows and heifers with living abortion bacilli is indefensible. In consequence of such inoculation the udder may become infected, with consequent risk to persons consuming the milk.

THE PRACTICAL VALUE OF THE HEAT-STABLE ANTIGEN OF *C. CHAUVÆI* AS AN IMMUNISING AGENT.

By R. S. ROBERTS,

South-Eastern Agricultural College, Wye, Kent.

IN endeavouring to find a satisfactory method of active immunisation against infection with *C. chauvæi* in sheep, the investigator is concerned with rather more than the preparation of an antigen which will confer adequate protection against natural infection, because the disease in most areas is either low or irregular in its incidence and it is therefore imperative that the agent used should be easily prepared and administered, definitely innocuous, and should remain unaltered when stored for relatively long periods. It appeared possible that many of these conditions might be fulfilled by a vaccine prepared entirely from the "O" or somatic component of *C. chauvæi* organisms. Robertson and Felix (1930) prepared a *V. septique* anti-serum by inoculation of a horse with the "O" antigen of that organism, and this serum appeared to afford some protection against inoculation with a whole culture of *V. septique*. In a previous paper (Roberts, 1931) it was shown that *C. chauvæi* organisms consisted of a heat-stable "O" antigen common to bovine and ovine strains, and a heat-labile "H" antigen specific to each type, ovine and bovine. Henderson (1932) reported the successful immunisation of guinea-pigs against *C. chauvæi* infection by inoculation with very large quantities of "O" antigen and expressed the view that the method might be of practical value in the active immunisation of animals in the field.

The objective of the work recorded here was to ascertain the value of such a vaccine in protecting sheep against *C. chauvæi* infection following wounding, but the data obtained during preliminary experiments upon guinea-pigs were insufficiently encouraging, and work upon sheep was abandoned.

Two vaccination experiments have been performed; in one, small doses of the antigen were used and the immunity was tested with a suspension of sporulating bacteria; in the second experiment massive doses of vaccine were used and the immunity was tested with whole culture.

Preparation of the "O" Antigen.

The standard technique, as described by Robertson and Felix (loc. cit.), were adhered to in all essentials. Two-litre quantities of 0.5 per cent. glucose broth under vaseline, of pH 7.5, in which were suspended two or three diffusion shells containing pieces of liver,

were inoculated with an actively growing 18-hour old culture of an ovine strain of *C. chauvæi*, an inoculum of 150 c.c. being employed. The strain used was isolated from a case of parturient gas gangrene in 1930, but its virulence and capacity for active growth in artificial media have been maintained by passage in sheep and guinea-pigs. Good growths were obtained, and distinct auto-agglutination was apparent after incubation for 24 hours. The culture was kept in a cold store for a few hours and the greater part of the supernatant was then removed by siphonage. The remainder was centrifuged at 2,000 revolutions per minute for 30 minutes and as much as possible of the supernatant removed. The deposit was re-suspended in distilled water and adjusted to an opacity equivalent to 37,870 millions of *B. coli* per cubic centimetre. This yielded a cream-like suspension. It was then heated at 100° C. for two hours and the saline concentration adjusted to 0.85 per cent. by addition of 10 per cent. salt solution. Sterile glass beads were introduced and the suspension vigorously shaken. The total volume of "O" antigen in this concentration obtained from two litres of culture was 70 c.c., or 3.5 per cent. of the original culture.

Method of Testing Immunity.

In previous investigations upon immunisation against *C. chauvæi* (McEwen and Roberts, 1932), when guinea-pigs have been used, the degree of immunity has almost invariably been estimated by intramuscular inoculation of a dose of culture which was known from experience to be almost invariably lethal, control guinea-pigs being inoculated in each experiment. As this dose is of necessity several times greater than is required to produce a lethal result in the majority of guinea-pigs, the method has the disadvantage of being too severe to detect slight immunity. In the experiments recorded here a lower dose has been employed, namely, one which is unlikely to cause complete mortality in groups of four or more. In the first experiment the test inoculation was prepared by leaving a broth culture of *C. chauvæi* in a cold store for 14 days, collecting the organisms by centrifuging and washing, and re-suspending to a standard opacity. It was then returned to the cold store and left for three to four weeks. When examined microscopically it was found to consist almost entirely of spores. Activation was obtained by adding 0.3 c.c. of a 5 per cent. solution of calcium chloride to each inoculum. When this was titrated in a small number of guinea-pigs irregular results were obtained (Table I).

TABLE I.
TITRATION OF LETHAL DOSE OF SPORE SUSPENSION.

<i>No. of Guinea-pigs.</i>	<i>Dose of Spore Suspension.</i>	<i>Result.</i>
3	0.3 c.c. of 1 in 100 dilution	+ + +
2	0.3 c.c. of 1 in 1,000 "	+ —
2	0.3 c.c. of 1 in 10,000 "	+ —

A 1 in 1,000 dilution was selected as the test inoculation.

In the second experiment a broth culture of *C. chauvoei*, which had been left in a cold store for several weeks, was used. This allowed a titration of the lethal dose to be made a few days before the culture was used to test immunity, as it is reasonable to suppose that any change which might take place in the culture after this period would be a slight one (Table II).

TABLE II.

TITRATION OF LETHAL DOSE OF OLD CULTURE.

No. of Guinea-pigs.	Dose of culture.	Result.
2	0.5 c.c.	+ +
3	0.25 c.c.	+ + —
3	0.1 c.c.	+ — —

Doses of 0.25 c.c. and 0.1 c.c. were used to test immunity. No significance is attached to the fact that two guinea-pigs succumbed to the dose of 0.25 c.c. and only one to the dose of 0.1 c.c., as it is known from experience that a dose of 0.5 c.c. of this type of culture is almost invariably lethal for guinea-pigs.

No attempt has been made to compare the two methods of producing artificial infection in guinea-pigs, but it is felt that no advantage attaches to the use of an artificial activator. Were it definitely established that the only effect upon the tissues of natural aggressin is the production of necrosis, then the inoculation of a known number of spores with a known quantity of activator would appear to be preferable to the use of an unmeasurable agent such as whole culture. In the present experiments the immunity has been stimulated by an antigen wholly devoid of aggressin, and it therefore appears unfair to avoid the inclusion of "aggressin" when estimating the degree of immunity.

EXPERIMENT I.

Four groups of four guinea-pigs were inoculated subcutaneously with a 1 in 5 dilution of the standard suspension of "O" antigen. Group 1 received two doses of 2.5 c.c. with a fortnight interval; Group 2 one dose of 5 c.c.; Group 3 two doses of 0.25 c.c. with a fortnight interval, and Group 4 a single inoculation of 0.5 c.c. The amount of culture to which these doses correspond are shown in Table III. An interval of 14 days was allowed for the development of immunity, which was determined by the spore suspension method. One guinea-pig in each group failed to resist the inoculation of a quantity of spore suspension which proved lethal in only two out of six control guinea-pigs. Vaccinated and control guinea-pigs which survived developed more or less severe lesions.

TABLE III.

No. of Guinea-pigs.	Vaccination.	Equivalent in Culture.	Test Inoculation.	Result.	Survival Period.
3 (one died during vaccination).	2.5 c.c. repeated after 14 days.	2 doses of 14.3 c.c.	0.3 c.c. of 1 in 1,000 dilution + 0.3 c.c. Ca Cl ₂ solution.	+ — — —	24 hours
4	1 inoculation of 5 c.c.	28.6 c.c.	ditto	+ — — —	36 hours
4	0.25 c.c. repeated after 14 days.	2 doses of 1.4 c.c.	ditto	+ — — —	60 hours
4	1 inoculation of 0.5 c.c.	2.8 c.c.	ditto	+ — — —	96 hours
6	—	—	ditto	+ + — — —	36 hours 36 hours
2	—	—	0.3 c.c. of 1 in 100 dilution of spore suspension.	— — —	
1	—	—	0.3 c.c. of CaCl ₂ solution.	— — —	

The test inoculation was in all cases made up to 1 c.c. with normal saline solution. The diagnosis was verified by observation of lesions and examination of smears from the local swelling and surface of the liver.

EXPERIMENT II.

Before proceeding further, it was decided to ascertain whether inoculation with massive doses of the "O" antigen could be relied upon to yield the results claimed. Twelve guinea-pigs were inoculated subcutaneously on three alternate days with 1 c.c., 2 c.c. and 2 c.c. of the undiluted antigen. One, a pregnant female, died seven days after the termination of the vaccination. Fourteen days after the termination of the vaccination five vaccinated and five unvaccinated control guinea-pigs were inoculated intramuscularly with 0.25 c.c. of old culture. The remaining six and six controls received 0.1 c.c. of the same culture. The results are shown in Table IV. Nine control guinea-pigs succumbed to the infection and two remained normal. Of eleven vaccinated guinea-pigs, three died, four developed extensive lesions, swellings without general symptoms were observed in three, and one remained normal. The diagnosis was verified as in the first experiment.

TABLE IV.

No. of Guinea-pigs.	Average Weight at Time of Administration of Test Inoculation.	Dose of Test Inoculation.	Result.
<i>Vaccinated—</i>			
5	484 grammes	0.25 c.c.	+ — — — —
6	570 "	0.1 c.c.	+ + — — — —
<i>Controls—</i>			
5	479 grammes	0.25 c.c.	+ + + + —
6	650 "	0.1 c.c.	+ + + + + —

SUMMARY AND DISCUSSION.

Two experiments have been performed to ascertain the value of the somatic antigen of *C. chauvæi* in immunising guinea-pigs against infection with that organism. In the first experiment the amounts of antigen used were arbitrarily chosen as (1) an amount which, having regard to its equivalent in culture, might be expected to confer some degree of immunity, and (2) the largest amount which it would be practicable to use in the field vaccination of sheep. Using these quantities no evidence of immunity was obtained. In the second experiment an amount of "O" antigen corresponding to 143 c.c. of whole culture was inoculated into each guinea-pig. Evidence of immunity was obtained, in that 72.7 per cent. of the vaccinated guinea-pigs, as against 27 per cent. of the controls, survived the test inoculation; the surviving vaccinated guinea-pigs, with one exception, showed moderately extensive lesions but recovered. In planning the first experiment, account was taken of the work of Henderson (loc. cit.), who reported that he had immunised nine guinea-pigs with large quantities of concentrated "O" antigen derived from an English ovine strain of *C. chauvæi* and that six and three of these survived inoculation with 1,000 and 100 lethal doses of spore suspension respectively. From this it appeared justifiable to assume that one fifth or even one-fiftieth of the quantities used by him might be expected to confer some immunity. This was not the case. In the second experiment the vaccination was conducted with strict adherence to Henderson's technique, except that in the preparation of the antigen the bacteria were not washed before re-suspension in distilled water. This step was omitted because the objective was the preparation of the antigen in the simplest possible manner, and the inclusion of a small amount of filtrate could not impair the immunising properties of the antigen. Of eleven guinea-pigs vaccinated with concentrated antigen one out of five succumbed to half the dose which is generally lethal for guinea-pigs, and two out of six succumbed to one fifth of that dose. The disparity between these results and Henderson's is considerable. It is confidently felt that there were no important differences in the immunisation process, and therefore the explanation must be searched for in the methods adopted for estimating the degree of immunity. Henderson states that the calcium chloride technique of artificial activation "gave a thoroughly reliable method for inducing regular infection with *C. chauvæi*." Henderson's method of testing immunity (by using 1,000 and 100 lethal doses) may therefore be considered to have been more drastic than that employed by me, where the test inoculation was not invariably lethal for the control guinea-pigs. It therefore cannot be contended that immunity was broken down by an excessive test inoculation, and it must be concluded that the method of vaccination does not confer as satisfactory an immunity as is claimed by Henderson. Furthermore, the inoculation of spore suspension activated by calcium chloride has not been found to be a more reliable

or constant method of producing a lethal result than the inoculation of whole culture, and no benefit is derived from employing spore suspension rather than whole culture in testing immunity.

Were the antigen used satisfactorily in all other respects the degree of immunity conferred by it upon guinea-pigs might have been regarded as sufficiently encouraging to warrant the extension of experimental work to sheep, but if, as appears probable, immunity can only be conferred by the use of large quantities of the antigen, and assuming that sheep would require as large a dose as guinea-pigs, its use upon sheep becomes impracticable in that to vaccinate a flock of 1,000 sheep 143 litres of culture would be required.

CONCLUSIONS.

Guinea-pigs can be afforded a moderate degree of immunity against artificial infection with *C. chauvæi* by inoculation with large quantities of the pure "O" antigen of the organism. The claim that this antigen confers almost complete immunity against 100 and 1,000 lethal doses of *C. chauvæi* organisms has not been confirmed, and doubt is cast upon the value of the artificially activated spore suspension as an agent for the estimation of immunity.

Immunisation of farm animals by "O" antigen alone is unlikely to be of practical value.

REFERENCES.

- Henderson, D. W. 1932. *Brit. J. Exp. Path.*, xiii, 421.
McEwen, A. D., and Roberts, R. S. 1932. *J. Comp. Path. & Ther.*, xlv., 212.
Roberts, R. S. 1931. *J. Comp. Path. & Ther.*, xlv, 246.
Robertson, M., and Felix, A. 1930. *Brit. J. Exp. Path.*, xi, 14.

ABSTRACT.

CONTAGIOUS ABORTION IN DENMARK.

By C. O. JENSEN.

THE regulations in force with regard to contagious bovine abortion in Denmark are as follows :—

In a suspected case of contagious abortion the owner of the herd, or his representative, is forbidden, for a time to be fixed by the Ministry of Agriculture :—

1. To send bulls, pregnant cows or heifers, or cows or heifers with a discharge from the uterus, to graze on common pasture ;

2. To sell bulls, cows, or heifers for other purposes than slaughter without informing the purchaser that the disease is occurring in the herd or to send such animals to a public auction ;

3. To allow bulls, cows or heifers to be used for service without informing the owner that the disease exists in the herd ;

4. To send cows or heifers for service at other places without notice that the disease exists in the herd.

In 1920, the disease was made notifiable and some regulations were imposed on infected herds. These, however, were of the nature of an experiment, and it was soon found that notification of the occurrence of the disease was to a large extent neglected, especially by the owners of the larger herds, which were often permanently affected.

In 1897, the regulations were revised and the obligation to report was abolished. Some of the changes were in the direction of weakening in respect of the least important points in the original regulations, but in others there was a strengthening with regard to the more important points.

The author finds it very difficult to say what has been the effect of these regulations.

The author's laboratory functions as the central laboratory of the Danish Veterinary Administration. The diagnostic examinations concerning abortion are made without cost to the owners, and in each year material from about 15,000 cases of abortion is sent to the laboratory. The present statistical material embraces more than 120,000 cases, but it has not yet been possible to tabulate the results or even to say whether the disease has increased or diminished during the last decade. It is thought, however, that the disease is less widely distributed than it was in former decades.

The measures employed to control the disease in individual herds have varied. Naturally, for this purpose early and strict isolation of the infected animals, as well as thorough disinfection, were recommended, but such precautions apparently were not very successful, probably on account of the readiness with which the infection spreads in an infected herd. For example, Axel Thomson, who carried out systematic examinations in 100 herds in which a case of abortion had occurred for the first time, found that blood tests of the whole of the animals within a few days afterwards indicated infection in about 65 per cent. of the animals. That immediate separation of infected cows and thorough disinfection may have a large measure of success in a case where the disease is of quite recent introduction is indicated

by the fact that in about 30 per cent. of the herds further cases of the disease did not occur. To what extent immediate separation and thorough disinfection are carried out in Denmark the author is unable to state, but he does not think that the methods can be regarded as satisfactory, owing to the difficulty of effecting a separation of the infected animals and in carrying out adequate disinfection.

In herds in which immediate removal of the cow that has aborted is not possible, or when several cases of abortion have already occurred, the removal of the aborted cows is not sufficient, and satisfactory results can only be expected through blood tests carried out at regular intervals, and isolation or complete removal of all the animals showing a positive reaction.

Some years ago experiments in the direction of dealing with outbreaks in this way were carried out, but they were abandoned as being impracticable for economic reasons.

In most cases in Denmark cases are not dealt with in any particular way; the owner sells infected animals and continues to hope that the plague will disappear spontaneously.

VACCINATION.

Shortly after the discovery of the abortion bacillus by Bang and Stribolt, some vaccination experiments were carried out on sheep, goats and cows. An account of these experiments was not published because the results were not free from doubt. The later investigations and experiments with regard to vaccination have been carried out under the guidance of two Institutes, namely, the Department for Special Pathology and Epizootiology of the Veterinary High School, and the Serum Laboratory which is attached to the same high school but also functions as a central laboratory of the Veterinary Administration.

Prof. Oluf Bang, Director of the first of these Institutes, has, for a number of years supplied to practising veterinary surgeons killed or living cultures of the bacillus for the purpose of vaccination. According to a report in 1922, about 3,300 animals had been vaccinated with this material, and the results were shown as follows:—

Of 772 animals vaccinated with living cultures, 126, or 16.3 per cent. aborted.

Of 624 animals vaccinated with killed culture, 248, or 39.7 per cent. aborted.

Of 1,889 animals vaccinated with living culture, 266, or 14.1 per cent. aborted.

The following separate figures were supplied with regard to heifers:—

Of 1,062 vaccinated with living culture, 153, or 14.4 per cent. aborted.

Of 589 inoculated with dead culture, 228, or 38.7 per cent. aborted.

Of 206 cows which had already aborted and were afterwards inoculated with living culture, 48, or 23 per cent. again aborted.

The author thinks that, as Prof. Bang was not able to have unvaccinated animals reserved as controls in these experiments, it is not possible to draw reliable conclusions from the results.

As long ago as 1909, experiments in the employment of vaccination against the disease were begun in the Serum Laboratory of the High School by the Director in collaboration with Prof. Holth and Prof. S. V. Wall. The experiments were carried out in the field, and both vaccine and serum were employed. However, neither at that time nor later was either serum

or vaccine sent out for use in general practice ; the treatment of the animals was undertaken exclusively by the Director's co-workers, and an extensive and faultless control material was always assured.

In the first experiments serum was used, and after that a combination of serum and bacillary extracts, partly with killed cultures ; later, killed cultures were exclusively employed. The results were occasionally strikingly good ; but critical examination of the whole material, which included 17 herds with from 1,000 to 1,100 experimental animals, made it clear that these methods of treatment were devoid of value.

Vaccination experiments with living culture were then undertaken. In order to have for the experiments animals as nearly as possible of the same character, only heifers that had not yet been served were employed ; they were selected in large strongly-infected herds where it was possible to have about the same number of experimental and control animals, and the author emphasises the fact that these two groups of animals were of equal value. The experiments embraced 447 experimental heifers and 424 control heifers. Of the experimental heifers, 23.7 per cent. aborted, and of the control heifers, 36.8 per cent. An accurate analysis of this experiment, however, shows the following :—

The first set of experiments (including 297 animals) was carried out in 1913-16, and with the result that 40 per cent. of the control animals aborted, but only 14.3 per cent. of the vaccinated animals. In 1916-17, the experiments embraced 354 animals and the result was less satisfactory, since 33.5 per cent. in the controls and 25.7 per cent. in the vaccinated aborted. In 1918-19 (220 animals) the result was bad ; 37.1 per cent. of the controls and 32.1 per cent. of the vaccinated animals aborted. The results of the experiments varied greatly in the different herds ; in some the number of cases of abortion was low in the vaccinated heifers in comparison with that among the control heifers (*e.g.*, 13.6 per cent. against 30 per cent. ; 8.3 per cent. against 25 per cent.). In other herds, however, there were a larger number of cases of abortion among the vaccinated than among the control animals (*e.g.*, 45 per cent. against 41.6 per cent., and 85.7 per cent. against 66.6 per cent.). The author here observes that much care is necessary in dealing with statistics.

As these results indicated that no great value could be attached to vaccination with living cultures, the author and his collaborators turned their attention to other aspects of the abortion question. They were, however, later induced to try still further experiments with living cultures in which the animals used were older cows and the vaccine was composed in part of so-called stable-specific cultures. The experiments were carried out in five herds and embraced 92 experimental animals, and 62 control animals. The results were bad, and showed that the vaccination had no protective effect.

The author observes that the *Brucella* infections are in many respects peculiar and that it seems not improbable that a closer study may bring to light some new and unexpected facts with regard to their immunity conditions. In one experiment it was possible to recognise what appeared to be a local tissue immunity. In one herd 30 non-pregnant heifers were selected ; 14 of these were given two separate injections of killed abortion culture into the uterus while the remaining 16 animals served as controls. In the same way in another herd 17 heifers were treated with living culture and 13 heifers remained as controls. The injections did not appear to have any injurious effect but they also produced no immunity.

In the first experiment three of the vaccinated and four of the unvaccinated animals, and in the last experiment four of the vaccinated and five of the unvaccinated, aborted. Some of the vaccinated animals and also some of the control animals did not become pregnant.

Personally, the author is of the opinion that vaccination with living cultures has not an immunising effect that would justify its general employment. The now recognised pathogenicity of the abortion bacillus for the human subject is calculated to raise great misgivings regarding such a procedure. (*Deutsche Tierärztliche Wochenschrift*. 1931. No. 48, pp. 733-735.)

REVIEW.

The Early History of Veterinary Literature and its British Development.

By the late Major-General Sir Frederick Smith, K.C.M.G., C.B. Vol. iv. The Nineteenth Century, 1823-1860. Edited, with a memoir of the author, by Fred Bullock, LL.D. London, 1933. Baillière, Tindall and Cox. Price, 15s.

This volume has been prepared from the manuscript written by the author during the last year of his life. He committed the task of completing his work and preparing it for the press to Dr. Fred Bullock, who had co-operated with Sir Frederick in the production of the first three volumes. This has obviously involved a large amount of labour in verifying biographical notes and checking references to dates and other matters in the author's manuscript. In some respects the present volume exceeds in interest the previous ones, because it deals with a period of outstanding importance in connection with the development of veterinary science and the veterinary profession in this country. It constitutes a record of all the contributions of any note that were made to veterinary literature in this country between the years 1823 and 1860. The authors of the period include names that are familiar in the profession down to the present time, including William Dick, Charles Spooner, J. G. Simonds, John Barlow, G. W. Varnell, and Sir George Brown.

The work is not a mere list of the articles on veterinary subjects that appeared during the period, but also contains a critical estimate of the value of the work of the different authors. As an example of the happy way in which the author has expressed in brief compass the character of the prominent men of the period, the following appreciation of Sir George Brown may be quoted: "As a lecturer he was dull and uninspiring, as a public speaker gifted beyond measure, impressive, severely logical, deliberate, and with a wide choice of language. He was not popular with students owing to his reserved manner, somewhat melancholy expression, and the extreme rarity of his smile, but he never failed to command respect. In private life, it is said that he showed an unsuspected sense of humour."

The frontispiece of the work is an excellent portrait of Sir Frederick Smith, produced from the oil painting by Dorofield Hardy in the Council Room of the Royal College of Veterinary Surgeons, and interleaved with the text of the work there are twelve portraits of the most eminent of the teachers and others of the period.

Dr. Bullock is responsible for an excellent memoir and a chronological list of Sir Frederick Smith's published books and papers.

It ought to be added that the publication of this volume was only made possible by the generosity of a few friends of the author, who provided the funds necessary for its publication in recognition of the immense debt that the profession owes to him.

Every member of the profession would do well to obtain a copy of the book.

ERRATA IN PART I.

1. *Legend of Fig. 8*, facing p. 9 : The last word should be " host," not " heart."
 2. *Page 10, para 3, line 6 :*
 - (a) Comma after " phagocytise " should be after " parasites."
 - (b) Insert " them " after " cut."
-

TO FOLLOW ON PAGE 41.

REFERENCES.

- Emmel, M. W. & Boevers, M. L. 1932. *J. Am. Vet. Med. Assoc.*, **81**, 92.
- Hughes, T. P. 1930. *J. Exp. Med.* **51**, 225.
- Mallman, W. L. 1930. *J. Am. Vet. Med. Assoc.* **77**, 636.
- Sheather, A. L. 1923. *J. Comp. Path. & Therap.* **36**, 255.

CONTENTS.

GENERAL ARTICLES.

	PAGE
The Control of Camel Trypanosomiasis. By S. C. J. Bennett, D.Sc., M.R.C.V.S.	67
Canine Distemper. The High Antigenic Value of the Virus after Photodynamic Inactivation by Methylene Blue. By J. R. Perdrau and C. Todd, F.R.S.	78
The Virus Diseases of Animals with Special Reference to those of Poultry. By T. M. Doyle	90
"Struck." Enteritis and Peritonitis of Sheep Caused by a Bacterial Toxin Derived from the Alimentary Canal. By A. D. McEwen	108
The Transmission of Louping Ill to Field Voles. By G. M. Findlay and C. Elton	126
Tumour of the Gall-Bladder in a Bullock. By Thomas Johnston, M.R.C.V.S., D.V.S.M.	129

NOTICE TO SUBSCRIBERS.

Communications, exchange journals, specimens, etc., for the Editor should be addressed to Sir John M'Fadyean, Highlands House, Leatherhead, Surrey.

BINDING CASES.—Maroon Full Bound Cloth. Can be obtained from the Publishers and Agents, Price 3s. 6d., post free.

PARÔPHYROXIÂ

(HEWLETT'S)

It possesses three distinctive therapeutic powers—i.e., Antiseptic, Purgative and Carminative—and is indicated in all cases of Colitis caused by undigested food, or irritative affections of the alimentary canal, partly no doubt by removing acrid irritating secretions or foreign materials, neutralising gaseous fluids, depleting the congested blood vessels, and arresting the distended and tympanitic condition of the colon, rendering it the safest and most satisfactory purgative in these cases. It is peculiarly adapted for those cases of Colic so frequently met with in aged horses, who are generally described as "Gross Feeders," where the food is bolted and not sufficiently masticated owing to the teeth being faulty, and where the digestive organs are impaired from a loss of tone in the system, the circulation bad, and the necessary amount of gastric juice is insufficient to digest the food. It is very extensively used in Scotland by numerous practitioners for the Colic caused by coarse feeding which produces impaction with painful contraction of the muscular coat of the bowels accompanied with tympanites.

It is in these cases that Parôphyroxiâ has a very marked effect in reducing the spasm, arresting fermentation, and subduing the drum-like condition of the colon. The large amount of clinical evidence adduced in its favour by various experienced and sound practitioners commends it so strongly that it is now placed at the head of the list of the different agents employed in the general treatment of Colic.

It should be well borne in mind that as the drug is purgative in its action no physic ball or other purgatives should be administered, but if the practitioner deemed it necessary to follow up the treatment with another draught, some simple antispasmodic would not be contra-indicated.

Price 4s. 6d. per lb. W. Quarts 4s. 3d. per lb.

SOLE AGENTS FOR THE VACCINES AND SERA OF THE BACTERIOLOGICAL INSTITUTE, TOURS.

Tuberculin (Subcutaneous, Intradermal and Ophthalmic), Mallein, Vaccines for Canine Distemper (Prophylactic and Curative), Pneumonia, Strangles in Horses, and Mammitis, etc. Pyovaccine for Sores and Contagious Lymphangitis.

ALSO AGENTS FOR THE JENSEN-SALSBERY BIOLOGICAL PRODUCTS,
PASTEUR INSTITUTE, PARIS, ETC.

Only Supplied to Members of the Veterinary Profession.

C. J. HEWLETT & SON, LTD.,

Wholesale and Export Druggists and Surgical Instrument Makers,
35-42, CHARLOTTE STREET, & 83-85, CURTAIN ROAD, LONDON, E.C.2.